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A MORPHOLOGICAL AND CYTOLOGICAL STUDY OF A NEW FORM OF VOLVOX—II*

JEROME METZNER

CYTOLOGY

In a very brief description of nuclear division in *Volvox minor* (*V. aureus*) Overton (1889) reported that the nucleolus is dissolved, the nucleus swells considerably and goes into a kind of spireme condition; division is described as of the indirect type involving the formation of thread-like chromosomes. Overton found that nuclear division in developing daughter colonies is synchronous. He considered lack of many division figures as indication that division occurs quickly. None of the details of nuclear division is described.

The first intensive account of vegetative mitosis, cell division and meiosis in *Volvox* was published by Zimmerman (1921). A summary of his observations follows: Before mitosis the nucleus and its enclosed nucleolus undergo enlargement, and the latter at times becomes vacuolated. The Randkörper, a small chromatic body usually found near the nucleolus, disappears during prophase. Condensation of chromatic material occurs and progresses until 12 chromosomes are formed. Up to this point the nucleolus remains structurally unchanged, but moves toward the nuclear membrane, gradually diminishing in size, and finally disappears as it reaches the nuclear border. In metaphase centrioles are sometimes visible at the poles of the intranuclear spindle. During telophase chromatic droplets appear between the disappearing chromosomes of each daughter set. Aggregation of chromatic materials results in the reformation of nucleoli and in addition the Randkörper reappears in each daughter nucleus. Cell constriction follows nuclear division. Zimmerman remarks that nuclear division must occur rapidly because division stages were found comparatively rarely. Material fixed at 3 p.m. on January 24, 1919, showed about 2 per cent of the nuclei dividing, and this is considered especially rich. No evidence of reduction division was observed in the formation of eggs or sperm, and every division of antheridial cells showed 12 chromosomes.

After a two-month resting stage, the oospore germinates, and the first nuclear division is reductional. Twelve bivalent chromosomes are produced; these form rings or tetrads in the center of the nucleus. From this point on Zimmerman admits that he does not have a complete picture of meiosis, but considers that the evidence is sufficient to warrant the conclusion that the

* Part I of this study appeared in Bull. Torrey Club 72: 86–113. 1945.

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zygote represents the diploid phase of the *Volvox* life cycle, and that reduction occurs during the first division of the germinating zygote nucleus. His figures seem to indicate an intact nuclear membrane throughout the mitotic cycle, although he does not mention that mitosis is intranuclear.

Studies on mitosis in *Volvox* are difficult because of the paucity of division figures. After colonies had been fixed at hourly intervals during the day and night, it was found that there were two periods of maximum mitotic activity—an optimum period, occurring at approximately 3 p.m., and another period with somewhat less activity taking place at 10 p.m. Colonies fixed at other hours of the day or night showed few or no signs of mitotic activity.

The interkinetic nucleus of an undivided gonidium (figs. 1, 98) is 13–17 μ in diameter and occupies a more or less central position within the cell. It is bounded by a definite membrane enclosing (a) a relatively large, spherical endosome, 4.5–6.7 μ in diameter, staining densely with iron-alum haematoxylin and giving a positive reaction to the Feulgen test; (b) a smaller, spherical body, 1–1.5 μ in diameter, usually located near the endosome, also staining with iron-alum haematoxylin, but reacting negatively to the Feulgen test; this nuclear element will be referred to as a plasmosome for reasons which are stated below; (c) a reticulum of delicate threads reacting with fast green counterstain; (d) occasional minute granules occurring within the meshes of the reticulum.

During interkinesis there is evidence that changes are occurring within the endosome. At times this body stains homogeneously with each of the nuclear stains used, but a process of vacuolization begins, during which, at first, numerous small clear, non-staining vacuoles are formed (figs. 2–5). As the vacuolization process continues, the smaller vacuoles seem to merge, resulting finally in an endosome with one or a few large vacuoles (figs. 3, 4). In two separate observations a pale, spherical endosomal bud was seen attached to the original endosome (fig. 90). The bud may be derived from vacuolar material, since the residual endosomal mass is non-vacuolated and smaller than non-budding, vacuolated endosomes, and the bud has the same negative staining reaction to nuclear dyes as the vacuolar material. The subsequent history of the endosomal bud could not be traced.

The onset of mitosis is usually made manifest by an accumulation of minute cytoplasmic threads, possibly mitochondria, around the nuclear membrane.

During prophase, the vacuolated endosome divides abruptly into two large, uneven masses which separate in opposite directions (figs. 6, 7, 99). This process must occur very swiftly, for few intermediate stages were observed between the splitting of the endosome and the arrival of the daughter halves at opposite poles of the nucleus (fig. 7). The endosomal substance

seems to have a high viscosity, for as the two daughter masses move apart to opposite poles, they remain connected for a time by many slender threads having numerous, bead-like condensations of endosomal material along their lengths (figs. 6, 99). These granular connecting threads disappear later, leaving two densely staining, polar endosomal masses completely separated (figs. 10–12). Frequently, endosomal division occurs in such a way that in addition to the two polar masses, smaller masses of endosomal material, one or several, become detached and remain near the center of the nucleus for a time (figs. 8–10), but disappear in later prophase.

Endosomal division followed by migration of the products to opposite poles of the nucleus usually prevails. No morphological mechanism for endosomal division could be detected. The spindle apparatus does not appear until late prophase. Infrequently, the endosome does not lie near the center of the nucleus but is eccentric. Such an endosome does not divide, but remains on the side of the nucleus even after the spindle has been formed (figs. 17, 91). There it gradually disintegrates and finally disappears.

During these early prophasic stages the plasmosome disappears completely and does not reappear until the daughter nuclei are formed. Because this body is Feulgen-negative, disappearing during prophase of mitosis and reappearing during late telophase, it seems to correspond in its general history to the plasmosome of higher plants.

Certain changes occur within the polar endosomal masses, resulting frequently in the formation of finger-like processes on the sides of the bodies facing the interior of the nucleus (figs. 8, 9). At the same time the outer portions generally begin to lose their avidity for nuclear dyes (figs. 8, 12, 106). While these changes are taking place the polar masses continue to move in their original directions and gradually push or dissolve their way through the nuclear membrane (figs. 10–13). During movement through the nuclear membrane into the cytoplasm those portions of the polar masses within the nuclear membrane stain densely with iron-alum haematoxylin especially at the inner edges, while the portions outside the nuclear membrane take only the fast green counterstain (figs. 12, 13).

Thus while extrusion is taking place chemical changes occur within the endosome. These processes continue until the polar masses are completely eliminated from the nucleus into the cytoplasm. There, at first, they are definite bodies not staining with iron-alum haematoxylin and reacting negatively to the Feulgen test. Shortly afterwards they disappear, leaving behind no trace in the cytoplasm (figs. 15, 16, 109, 110, 111). A spindle appears in the plane occupied previously by the connecting strands of the polar masses (figs. 15, 111, 112). Prophasic chromosomes showing evidence of equational division and apparently derived from elements within the nuclear reticulum, are formed (fig. 14) and arrange themselves on the equator of the spindle (fig. 100).

Because metaphase figures occur with relatively high frequency it may be assumed that this phase is retained for a longer period than some of the others. At the beginning of anaphase there may be some faint evidence of intranuclear polar masses (figs. 15, 109). Later, however, when the daughter chromosome-sets separate there is no trace of intranuclear endosomal material (fig. 16). Considerable shrinkage occurs in the chromosomes between prophase and metaphase so that at the latter stage the chromosomes are finally about 0.5 μ or smaller in diameter, whereas formerly they were approximately 0.75–1.0 μ. Because of their extremely small size and crowding it was impossible to determine the number of the chromosomes. In figure 14 there are 20 bodies, some of which are obviously double whereas others may or may not be so. The preceding section through the same cell contains two more bodies, giving a total of 22. However, since the slide was stained with iron-alum haematoxylin it is impossible to determine which of the bodies are chromosomes and which are endosomal fragments. None of the Feulgen-tested material had prophasic stages with large divided chromosomes, but it contained a large number of metaphases with chromosomes too minute and closely packed to permit an accurate count.

Few anaphases were observed, indicating that movement of the chromosomes from the equator of the spindle to opposite poles occurs rather quickly (figs. 15, 16, 17, 111, 112).

After the chromosomes have reached the opposite poles of the spindle (figs. 18, 113) they begin to aggregate there. A rather densely staining coarse reticulum is formed in which the identity of the separate chromosomes is lost (figs. 19, 114, 115, 118). The total amount of chromatic material seems to increase. These tendencies, aggregation of chromatic masses together with increase in total amount of chromatic material, continue until finally two large compact spherical endosomes are formed within separate nuclei (figs. 20, 119). In addition the plasmosome makes its appearance at about this time. All of these mitotic processes appear to occur within an intact nuclear membrane. Although final separation of two nuclear masses was not observed it seems probable that the nuclear membrane between the chromosome-sets constricts, and is eventually severed near the center, thus producing two nuclei within the dividing cell (figs. 116, 119). Karyokinesis definitely precedes cytokinesis, yielding a binucleate stage which is maintained for some time (figs. 20, 116, 119). Later, the cytoplasm begins to constrict in the region between the two daughter nuclei (fig. 116). Constriction continues until cytokinesis is completed. Newly formed nuclei are considerably smaller (fig. 116) than older nuclei which are about to divide. As newly formed daughter cells of young developing daughter colonies carry on their metabolic activities their nuclei increase in size and may reach about 17 μ before undergoing mitosis.

Mitosis in *V. Carteri* is intranuclear and it seems likely that this condition also obtains in *V. aureus* (Zimmerman 1921). Both species have similar resting vegetative cell nuclei each possessing a large endosome and an additional body called a Randkörper by Zimmerman, but interpreted in this paper as a plasmosome. In each species this body disappears during the prophase of mitosis. On the basis of certain microchemical tests Zimmerman (1921) held that the nucleolus of *V. aureus* was similar to that of higher plants. However, the Feulgen test (Feulgen & Rossenbeck 1924) was not available at that time and relatively little was known of the nucleic acid content of nuclear elements or of the cytoplasm. Beyond the similarities already indicated between the vegetative cell nuclei of *V. Carteri* and *V. aureus* there are few additional ones once division begins. Endosomal division, characteristic of cell division in *V. Carteri* does not occur in *V. aureus;* nor does Zimmerman report nucleolar budding or extrusion. In *V. aureus,* he observed centrioles at the poles of many metaphase spindles, but they could not be identified with certainty in *V. Carteri.* The latter species has minute granular chromosomes of indeterminate number whereas in *V. aureus,* the chromosomes are considerably larger and thread-like.

Thus there are several outstanding differences in the mitotic cycle of these two species of the genus *Volvox.*

Since it has been impossible, thus far, to secure division figures in germinating oospores of *V. Carteri* no statement can be made concerning any changes in chromosome number which may occur. However, the fact that some germinating oospores were found containing from two to four nuclei, is at least suggestive of the possibility that in some oospores meiosis takes place. Moreover, the large number of germinating oospores having single nuclei, together with the evidence previously presented in this paper, also suggests that these uninucleate germinating oospores are haploid parthenospores. Pocock (1933b) also observed a few oospores with their contents divided into four and states that in germination of the oospore reduction probably takes place.

Among the protista intranuclear mitosis, involving a large nucleolus-like body, is common. Wilson (1925) and Sharp (1934) mention many examples of this type of mitosis. Wilson (1925) states that in such cases "an intranuclear spindle is formed by elongation and ultimate division of the karyosome." "The poles of the spindle in typical cases are occupied by deeply staining 'polar masses' or 'polar caps' derived directly or indirectly by division of the karyosome. The spindle itself appears to be formed in some cases entirely from the karyosome, in other cases in part at least from the linin substance of the peripheral nuclear zone surrounding the karyosomes." In its broad features this description of protistan mitosis characterizes mitosis in *V. Carteri,* but bears little relation to the mitotic cycle of *V. aureus* de-

scribed by Zimmerman (1921). The extrusion of the endosome-derived polar masses into the cytoplasm is an unusual additional feature of mitosis in *V. Carteri*. Concerning the mechanism responsible for division of the endosome and the origin of the spindle, little can be said on the basis of actual observation. It is possible that division of the endosome is autonomous. Progressive vacuolization in this body indicates that it is not a passive nuclear structure. The endosome may function as a centriole or contain within it a centriole although no evidence of such a structure could be detected by any staining technic. There is also the possibility that a physico-chemically differentiated, but morphologically undifferentiated spindle apparatus is responsible for the rapid division of the endosome into polar masses. That this region usually develops near the central axis of the nucleus is indicated by those nuclei in which the endosome is situated eccentrically. Under these conditions the endosome is not divided but remains on the side of the spindle which is formed later. Finally, there is the possibility that formation of polar masses is due to a combination of the two forces indicated above, namely, autonomous division of the endosome together with the presence of an invisible, but functional spindle mechanism.

Superficially it would appear that the connecting strands between the polar masses become converted into the spindle mechanism. However these threads stain with iron-alum haematoxylin, are granular, and seem to disappear. Shortly afterwards a typical spindle appears in the same plane as the connecting strands. If the possibility suggested above is true, this spindle apparatus has been present in the nucleus from the beginning of mitosis. It may be responsible for endosomal division resulting in the formation of polar masses and their connecting threads. Then this hitherto invisible mechanism becomes morphologically differentiated into an orthodox spindle.

Frew and Bowen (1929), describing mitosis in a cucurbit, show that the accidental location of the nucleolus in the spindle area results in the elongation of the nucleolus with its long axis parallel to that of the developing spindle and finally its separation into two masses. Normally in the cells of the cucurbit the nucleolus is to one side of the developing spindle and there undergoes dissolution. This condition is similar in some respects to that which obtains in *V. Carteri,* in which the endosome usually is caught in the spindle area and divided, but when the endosome is at one side it remains undivided and undergoes gradual dissolution. In each case the determining factor of nucleolar or endosomal division is occurrence of these bodies in the spindle area—fortuitously or normally.

Nucleolar extrusion in animals correlated with the elaboration of cytoplasmic products such as yolk and fat has been summarized recently by Gates (1942). Except in certain fungi, nucleolar division and extrusion among the protista are not common.

It is generally recognized on the basis of Feulgen staining that the nucleoli (plasmosomes) or related structures of most plant and animal cells do not contain chromatin. However, a few organisms in which such bodies have been found to be Feulgen-positive are cited by Gates; e.g., the nucleolus in the egg of *Stegomyia* and the periphery of the nucleolus of *Anopheles* (Bauer 1933). In *Ceratomyxa* Noble (1941) finds that the karyosome is Feulgen-positive. Thus, although the number of cases is relatively few, there is definite evidence that some nucleoli or related structures are Feulgen-positive, which indicates that they contain desoxyribose nucleic acid.

White (1942), summarizing the research of the principal workers in the field of nuclear chemistry, states some of the known facts concerning nucleic acids as follows: "Nucleic acids are polymers of nucleotides each nucleotide being composed of one molecule of phosphoric acid, a pentose sugar and a purine or pyrimidine ring. The various kinds of nucleotides have different purines or pyrimidines. Apart from this there are two main types of nucleotides which differ in the nature of the pentose. In one type the sugar is d-ribose while in the other it is desoxyribose. The Feulgen reaction depends on the Schiff reaction for aldehydes which is given by the desoxy—but not by the ribose sugars."

Caspersson (1936, 1940) developed an ultra-violet absorption technic for detecting nucleic acids, and using this method, demonstrated the presence of large quantities of nucleic acids in the nucleus and cytoplasm of cells about to undergo rapid division. A region that strongly absorbs ultra-violet light at 2600 Å is assumed by Caspersson to contain nucleic acid. If it is Feulgen-positive the nucleic acid is assumed to be the desoxyribose type. If Feulgen-negative it is considered to be the ribose type. Extensive studies by the Caspersson school have shown that nuclear chromatin is always rich in desoxyribose nucleic acid; while the nucleic acid of the cytoplasm is of the ribose type. It has been established that true nucleoli (plasmosomes) contain ribose nucleotides, and it is believed that they may represent temporary storage places for nucleic acid. Because of these important differences in distribution of the two nucleic acids it has been suggested recently that desoxyribose nucleic acid be called chromonucleic acid and that the ribose form be called plasmonucleic acid (Pollister and Mirsky 1943).

There have been several observations which suggest that the nucleic acids may change their position from nucleus to cytoplasm (Schultz 1941) or vice versa (Painter 1943) with a coincident alteration to the type of nucleic acid characteristic of the new location. By analogy with these when a Feulgen-positive material goes from the nucleus to the cytoplasm and there becomes Feulgen-negative there is ground for the assumption that this involves the conversion of a nuclear chromonucleic acid to its cytoplasmic counterpart, plasmonucleic acid.

In *V. Carteri* a Feulgen-positive endosome is divided into two polar masses, each Feulgen-positive, which are extruded into the cytoplasm, and there become Feulgen-negative and ultimately lose their identity. Only a few division figures showing the extruded polar masses in the cytoplasm have been found. However the history of the endosomal masses presented above suggests that desoxyribose nucleic acid stored in the massive endosome is extruded into the cytoplasm and there undergoes a change to some other substance—possibly plasmonucleic acid. It is difficult to speculate on the function of the extruded nucleic acid in the cytoplasm except to suggest a possible role in the elaboration of cytoplasmic storage materials found in abundance in the cells of developing colonies (Schultz 1941).

The difficulties experienced by Overton (1889) and Zimmerman (1921) in finding division figures were also shared by the writer. Although two daily periods of maximum division were found, the percentage of division figures was far less than the 2% found by Zimmerman.

Synchronization of division observed by Overton and Zimmerman in other species also occurs in *V. Carteri*, even up to well advanced stages in the development of daughter colonies.

TAXONOMIC CONSIDERATIONS

After the discovery of *Volvox* by Leeuwenhoek (1700), two species, *V. globator* and *V. aureus*, became known in both the Old and New Worlds. Further studies increased the number of known species to ten according to Pascher (1927) and seventeen according to Printz (1927). New species and several forms of some of these species have been described since these reviews, so that some 15–20 species of *Volvox* are known to date. In the United States the following species have been reported up to the present time:

With protoplasmic connections: *V. globator* (L.) Ehrenberg, *V. perglobator* Powers, *V. aureus* Ehrenberg.
Without protoplasmic connections: *V. Weismannia* Powers, *V. spermatosphaera* Powers, *V. mononae* Smith.

Carter (1859) found a species of *Volvox* in Bombay, India, which he erroneously called *Volvox globator*. After reading Carter's account, Stein (1878) corrected the error by renaming the species *Volvox Carteri*. Powers (1908) described three new species of *Volvox* from Nebraska, including *V. Weismannia*. Various Philippine forms of *Volvox*, including *Volvox Carteri* var. *Manilana*, were reported by Shaw (1919, 1922a, b, c). He pointed out that the strong similarities existing between *V. Carteri* and *V. Weismannia* warranted their inclusion in one species. *V. Weismannia* became *V. Carteri* var. *Weismannia*. Printz (1927), summarizing the genus *Volvox* in Engler's "Die natürlichen Pflanzenfamilien," placed *V. Carteri* in the *Merrillosphaera* section of the genus, and, in addition, followed Shaw by including

V. Weismannia as a variety of *V. Carteri*. Pascher (1927) stated that *V. Weismannia* was an uncertain species which Shaw included with *V. Carteri*—itself a very inadequately known form. Playfair (1918) found *V. Carteri* in algal collections made in Australia. The type form of *V. Carteri* as well as *V. Carteri* f. *nagariensis* were reported by Iyengar (1933) from Madras, India. Apte (1936) observed some species of *Volvox* including *V. Carteri* from Poona, India. A new variety of the species was not created, although his description seems to warrant it. The close relationships as well as the differences between *V. Carteri* and *V. tertius* are indicated by Pocock (1938) in her paper on the latter species. Thus *V. Carteri* has been found in India, the Philippine Islands, Australia, and in Nebraska.

In the summers of 1941, 1942, and 1943 the writer collected *Volvox Carteri* from aquatic plant tanks at Saddle River, Bergen County, New Jersey. This form of *Volvox* was available from early May to September in enormous quantities, free from other species of *Volvox*. Certain constant minor variations in size of the coenobia and oospores, membrane structure, and particularly the number of oogonia and sperm platelets observed over a period of three years seem to be sufficient to distinguish *Volvox Carteri* var. *Hazeni*,[3] the distinguishing characteristics of which are as follows:

Volvox Carteri var. **Hazeni** Metzner, var. nov. Coloniae vegetativae 500–756 μ × 500–785 μ metientes, e 4000–5000 cellulis globosis vel ellipsoidalibus vel leviter ovoideis constitutae; membrana communi protoplasmata e globosis ad ellipsoidalia a filis non nectata prope peripheriam claudenti; quaque cellula in prismate 5–7-laterali gelatinoso conclusa membrana exteriori convexa interiorique leviter orbiculata munito. Propagatio asexualis pro more 8 colonias filiales confingendo, eorum cellulis sexualibus cum 64 cellulae adsunt distinctis atque mensuram magnam ante divisione attingentibus; cellulae sexualis protoplasmate in membrana presse adhaerenti et in prismatis membrana ab eo leviter separata concluso; coenobii cavea materia alveolata gelatinosa atque cellulorum vegetativorum sexualiumque membranis prismaticis expleta.

Coloniae sexuales rigide heterothallicae; coloniis parvis masculinis 242–357 μ × 257–364 μ metientibus e 600–1100 cellulis constitutis quorum circa 50 discos antherozoidiorum leviter cupuliformes facientibus; disco plerumque 128 antherozoidia foventi post coloniam masculinam e coenobia parentali expulsam maturescentia; coloniis femineis maturis 371–671 μ × 392–678 μ metientibus quam coloniis asexualibus maturis majoribus e 3500–4000 cellulis constitutis; colonia 18–20 oosphaeras foventi ut oosporas maturescentes exospora reticulata munitas atque protoplasmata centralia in maturitate fulva habentes; aplanosporis vel zoosporis biflagellatis ex oosporis germinantibus orientibus.

Coloniae parentales colonias asexuales, masculinas, femineasve vel harum combinationes capientes, at masculinis femineisque nunquam in eadem colonia parentali praesentibus.

[3] In honor of the late Professor Tracy E. Hazen.

In his description of *V. Carteri* Carter (1859) pointed out that male coenobia contained about 100 sperm platelets and that female colonies contained 30–50 eggs. In *V. Carteri* var. *Hazeni* male coenobia have about 50 sperm platelets and there are usually 18–20 eggs in the female coenobia. These are the major distinguishing features of the new variety.

A chart summarizing the pertinent data published by those who have worked with *V. Carteri* has been constructed to include, under selected categories, similar information on *V. Carteri* var. *Hazeni*. Data on the other species of the section *Merrillosphaera* have been included for comparison. Blank spaces in the table represent gaps in the knowledge of these forms (Plate 1).

A diagram representing the life cycle of *V. Carteri* var. *Hazeni*, as described in this account, is included (fig. 120).

Summary of Numerical Data

Asexual colony	500 μ to 756 μ × 500 μ to 758 μ
Sexual colony, male	242 μ to 357 μ × 257 μ to 364 μ
Sexual colony, female	371 μ to 671 μ × 392 μ to 678 μ
Somatic protoplast, median optical view	6.7 μ to 8.3 μ
Length of flagella	21.7 μ to 23.4 μ
Asexual colony, just before birth	207 μ to 236 μ × 214 μ to 243 μ
Male colony, just before birth	228 μ to 257 μ × 235 μ to 263 μ
Female colony, just before birth	207 μ to 236 μ × 214 μ to 243 μ
Gonidium, before dividing	about 80 μ
Egg	48 μ
Oospore	about 54 μ; 46–60 μ
Protoplast of oospore	about 45 μ
Number of cells, asexual colony	4000–5000
Number of cells, male colony	600–1100
Number of cells, female colony	3500–4000
Number of daughter colonies	characteristically 8
Number of oospores	18–20
Number of sperm platelets	about 50
Sperm platelet	30 μ to 33.4 μ × 11.6 μ to 14.1 μ
Number of spermatozoids per platelet	128
Attachment of flagella on spermatozoids	terminal

Various criteria for distinguishing *Volvox* species have been established by different workers throughout the world. Meyer (1896) showed that the internal structure and membranes of *Volvox* colonies, as revealed by his methylene blue technic, were features which could be used together with the more familiar ones in distinguishing *Volvox* species. Shaw (1922a, b, c) thought that membrane and internal structure of *Volvox* colonies stained by Meyer's technic, along with the following additional features, were important: 1. Presence or absence of protoplasmic connections; 2. The stage at which the gonidia differentiate. However, Pascher (1927) states that differences among *Volvox* species such as the stage of differentiation of gonidia depend, to a large extent, on external conditions and thus do not have universal application. He believes that metabolic rate, temperature, and pH play the greatest role in determining certain structural and developmental features of *Volvox* and he has developed a theory of the polyphyletic origin

	Gonidia No. & Distribution	Max. Size	No. of sperm bundles	No. of spermatozoids per bundle	Shape of coenobium	Somatic protoplasts shape	Somatic protoplasts size	Sex of coenobia	Number of cells in coenobia	Eggs	Oospores	Size
V. Carteri Stein	generally 8 arranged in tetrads (Shaw)	85μ (Shaw)	100 or more scattered all over the periphery	128	globose, i.e. spherical or nearly so	globose-ovoid	...	heterothallic	not estimated	30-50 located in posterior 3/4 of colony	thickened wall with slightly wavy margin	40μ
V. Carteri var. Weismannia (V. Weismannia Powers)	most commonly 8 and 10 predominantly 10	90μ	number of sperm plates great in relation to vegetative cells	64 and 128 side by side	spherical or nearly so (Shaw)	.....	...	heterothallic	2500 to 7500 (Shaw)	18 to 20 (Shaw)	.....	...
V. Carteri var. homothallicus (Gonium bellosphaera obversa Shaw)	8, 7 or 6, sometimes fewer; arranged in tetrads and/or pairs	50 to 90μ	less numerous than oogonia	128	spheroidal - more or less elongate	globose or ovoid encased in gelatinous prisms with truncated anterior and much rounded posterior ends	...	homothallic	2000 to 3000	.....	outer wall reticulately wrinkled or ridged	32 42μ
V. Carteri var. Manilana Shaw	8, symmetrically arranged in tetrads. Sometimes 1 to 4 additional posterior ones	85μ	112	128	spherical or slightly elongated	ovoid	5 to 6μ	heterothallic	asex.: 11,400 female: 8000 male: 2500	24 to 40 posteriorly placed	slightly wavy wall; eccentric protoplast	50
V. Carteri from Madras Iyengar	3 to 8 usually 4 to 6	...	about 75 occupying all except extreme anterior end	...	globose to sub-globose	cells pentagonal or hexagonal in surface view	width 4.5 to 5.5μ height 5.5 to 6.5μ	heterothallic	asex.: 3000 to 11,000 female: 2000 to 3000 male: 500 to 700	17 to 29	smooth inner and wavy crenate outer wall	47 47
V. Carteri var. Nagariensis Iyengar	sometimes as many as 21, usually 15	...	...	...	globose	round in surface view, pear-shaped in side view	width 6 to 7μ length up to 9μ	heterothallic	8000	.....	.....	..
V. Carteri from Poona Apte	generally 8 to 12 arranged in pairs or fours	up to 21μ	more than 75 or near 100	...	more or less globose	roundish in outline as seen from the surface	6.7 to 8μ in diam.	heterothallic	3200 to 4500	30 to 35	outer wall wavy in outline neither smooth nor spiny	47 47
V. Carteri var. Hazeni	characteristically 8 arranged in alternating tetrads	about 80μ	about 50 scattered over periphery	128	more or less globose	globose-ovoid	6.7 to 8.3μ	heterothallic	asex.: 4000 to 5000 female: 3500 to 4000 male: 600 to 1100	18 to 20 range from 8 to 23	outer margin slightly wavy in outline, surface reticulately wrinkled; protoplast centrally placed	ab 4 8
V. tertius Meyer	2 to 12 most often 3 to 8	30 to 40μ	1/4 to 1/3 of the cells of a male colony	32 rarely 16	sub-globose to ovoid	rounded	6 to 9μ x 7 to 9μ	heterothallic	asex.: 500 to 2000 female: 500 to 2000 male: 180 to 512	2 to 11 most often 3 to 8	thick smooth exospore and thinner endospore surrounding the eccentrically lying protoplast; exospore has an irregular inner surface	20 20 20
V. africanus West	2 to 8 in pairs	28 to 44μ	100 to 400	either 64 or 128 (Shaw)	strongly ovoid to ellipsoidal	rounded	5 to 7μ	mostly heterothallic sometimes homothallic	asex.: 3000 to 7000 female: no data available male: no data available	12 to 80	thick and smooth	
V. gigas Pocock	up to 82 commonly 30 to 60	35 to 49μ	up to about 600 or 700	variable; most often 32 but also 16, 64, 256, 512 or more	sub-globose	rounded	9 to 13μ	heterothallic and mixed (i.e. asexual and sexual)	1000 to 3000	up to about 300 or 400	thick smooth exospore	

...ic protoplasts ... size	Sex of coenobia	Number of cells in coenobia	Eggs	Oospores	Size	Size of unborn coenobia asexual	male	female	as
...-ovoid ...	heterothallic	not estimated	30-50 located in posterior 3/4 of colony	thickened wall with slightly wavy margin	40µ	192µ	...	...	77 744
....	heterothallic	2500 to 7500 (Shaw)	18 to 20 (Shaw)	.....	...	more than 190µ	...	...	664 (1 oth 580
...s or ovoid ...d in gel- ...s prisms ...runcated ...ar and ...ounded ...ier ends ...	homothallic	2000 to 3000	.....	outer wall reticulately wrinkled or ridged	32 to 42µ	200µ	...	...	le
5 to 6µ	heterothallic	asex.: 11,400 female: 8000 male: 2500	24 to 40 posteriorly placed	slightly wavy wall; eccentric protoplast	50µ	200µ	...	...	72
...tagonal ...onal in ...view width 4.5 to 5.5µ height 5.5 to 6.5µ	heterothallic	asex.: 3000 to 11,000 female: 2000 to 3000 male: 500 to 700	17 to 29	smooth inner and wavy crenate outer wall	42 to 47µ	...	...	...	46 48
...surface ...ar-shaped ...view width 6 to 7µ length up to 9µ	heterothallic	8000	.....	.....	....	...	...	...	10 di
...in cut- ...seen from ...ace 6.7 to 8µ in diam.	heterothallic	3200 to 4500	30 to 35	outer wall wavy in outline neither smooth nor spiny	42 to 45µ	up to 198 x 231µ	238 x 253µ	...	2 8
...ovoid 6.7 to 8.3µ	heterothallic	asex.: 4000 to 5000 female: 3500 to 4000. male: 600 to 1100	18 to 20 range from 8 to 23	outer margin slightly wavy in outline, surface reticulately wrinkled; protoplast centrally placed	about 54µ 46 to 60µ	207 to 236µ x 214 to 243µ	228 to 257µ x 235 to 263µ	207 to 236µ x 214 to 243µ	5 5
6 to 9µ x 7 to 9µ	heterothallic	asex.: 500 to 2000 female: 500 to 2000 male: 180 to 512	2 to 11 most often 3 to 8	thick smooth exospore and thinner endospore surrounding the eccentrically lying protoplast; exospore has an irregular inner surface	58 to 66µ most often 61 to 63µ	115 to 163 µ x 135 to 480µ	...	...	2 3
5 to 7µ	mostly heterothallic sometimes homothallic	asex.: 3000 to 7000 female: no data available male: no data available	12 to 80	thick and smooth	43 to 45µ	...	...	...	3 3
9 to 13µ	heterothallic and mixed (i.e. asexual and sexual)	1000 to 3000	up to about 300 or 400	thick smooth exospore	31 to 44 µ	108 to 338µ x 129 to 431µ	...	...	1 1 1 1 1

coenobia female	Size of adult colonies asexual	female	male	Remarks
...	770µ 744 x 765µ (Shaw)	606µ (largest) 606 x 616µ (Shaw)	270µ 283 x 287µ (Shaw)	Carter's diagrams showed the absence of protoplasmic connections. Shaw (1922b) interpreted Carter's data.
...	664 x 672µ (largest - Shaw) others 580 x 605µ	.....	210µ (Shaw)	Powers did not describe female colonies. Shaw (1922b) interpreted Powers' data.
...	less than 500µ	.....	.....	The gonidia of this form of Volvox were said by Shaw to migrate from outside to inside the embryo through the phialopore. This change of position is now known to be brought about by eversion.
...	720 x 760µ	690 x 730µ	260 x 280µ	
...	460 - 630µ x 485 - 646µ	sub-globose up to 578 x 612µ	ellipsoid to globose 221 x 234µ	Iyengar states that this form of Volvox is identical with that described by Carter.
...	1003µ in diameter	.....	.....	Only the asexual coenobia of this form were found.
...	264 x 231µ to 891 x 729µ	unborn 504 x 455µ to adults 245 x 210µ	unborn 238 x 252µ to adults 126 x 140µ	
207 to 236µ x 214 to 243µ	500 to 756µ x 500 to 785µ	371 to 671µ x 392 to 678µ	242 to 357µ x 257 to 364µ	Of the colonies about to be born the males are slightly but constantly larger. Sperm plates mature after the male colonies are born.
...	280 to 559µ x 302 to 590µ	258 to 409µ x 311 to 480µ	129 to 185µ x 139 x 173µ	Each cell has a dome-shaped membrane. Reproductive cells may give rise all to infant colonies or all to eggs or occasionally some to colonies and some to eggs. Eggs and colony initial cells are difficult to distinguish.
...	366 to 926µ x 387 to 1077µ	slightly smaller than asexual colony; bisexual colony same as above	much smaller than asexual colony	Sexual material scarce hence the lack of information on it. Membrane structure as in V. tertius except 1. adjacent cell membranes are often separate from one another. 2. There is a distinct inner limiting membrane Androgonidia (sperm initials) divide at about the time of birth of the coenobium or afterwards.
...	1615 to 2800µ x 1700 to 3135µ mixed colonies of similar dimensions	1679 to 2154µ x 1723 to 2206µ	732 to 1141µ x 797 to 1292µ	In the posterior part of the male colony all cells become androgonidia - they are not mixed with somatic cells. The membranes are similar to those of V. tertius and V. africanus but adjacent cell membranes usually separate from each other. There is a distinct inner limiting membrane. Some coenobia are mixed; i.e. contain asexual and sexual reproductive cells.

of the genus based largely on a consideration of the cell and colony membrane structure. Iyengar (1933) considers that apart from cell structure the following criteria are fundamental in taxonomic considerations: 1. The distribution of the sexes; 2. The numbers of reproductive cells; 3. The disposition of the gonidia; 4. The character of the oospore. Pocock (1938) lays great stress on membrane structure as a basis for specific segregation in *Volvox*.

Most of the recent workers agree that an application of methods such as those of Meyer (1896) to a study of the details of cell structure and internal anatomy is essential for a clearer understanding of the taxonomic relationships in the genus *Volvox*. Most of the criteria stressed by various workers seem to be valid. Further comparative studies especially on the germination of the oospore and cytological analysis of various *Volvox* species will undoubtedly provide additional useful criteria.

The differences existing among species of *Volvox* seemed great enough to Shaw (1922a) to warrant their separation into different genera. Shaw's ideas on the reclassification of *Volvox* were incorporated in Printz' (1927) treatment in which, however, Shaw's generic names were given to sections of the one genus, *Volvox*. It is interesting to note that Printz included the section *Campbellosphaera* containing *Volvox obversus* (Shaw) Printz. Pascher (1927) and Iyengar (1933) recognize Shaw's error in distinguishing this form of *Volvox* as a separate species. In the opinion of Iyengar (1933) and Smith[4] (1933) the establishment of separate genera by Shaw seems unwarranted. Fritsch (1935), Pocock (1933), and Pascher (1927), however, have tentatively accepted Printz' treatment of the genus pending further investigations.

Inversion in *Volvox* was first noted by Powers (1908) and later described, in detail, by Kuschakewitch (1922), Zimmerman (1925) and most completely by Pocock (1933b, 1938). Since Shaw (1919) was apparently unaware of the inversion process in *Volvox* he incorrectly established the genus *Campbellosphaera* based on features which are now recognized as inversion phenomena. In view of these considerations it is apparent that the section *Campbellosphaera* of the genus *Volvox* is unwarranted and the form described by Shaw as *Campbellosphaera obversa* should be renamed and placed in its proper taxonomic position, namely, as a variety of *Volvox Carteri*.

It becomes evident, after a review of the more important taxonomic literature on *Volvox*, that a revision of the genus is needed. The acceptance, by several of the more recent workers, of Printz' division of the genus into sections indicates that this treatment has merit. In the writer's opinion it is a convenient device which can be improved as further investigations on all species of *Volvox* establish sound diagnostic features for each section.

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[4] Smith, however, incorrectly states that *V. Weismannia* Powers possesses stout protoplasmic connections.

Printz (1927) characterizes the section *Merrillosphaera* as follows: protoplast spherical-egg shaped, lacking protoplasmic connections; gonidia developed early in ontogeny and reaching a good size before cell division is initiated. All descriptions of the species of *Volvox* included in the section mention these features and therefore the section *Merrillosphaera* seems to have some validity. Printz included in the section *V. africana* West, *V. Carteri* Stein [= *Merrillosphaera Carteri* (Stein) Shaw incl. *V. Weismannia*], *V. Migulae* (Shaw) Printz, *V. tertius* Meyer, and *V. mononae* Smith.

Pocock (1938) believes that *V. Migulae* is identical with *V. tertius*, and both she and Pascher (1927) agree that *V. mononae* is not a good species.

It seems advisable to abandon *V. mononae* (Smith 1920) as a species of *Volvox* and to allocate *V. Migulae* to the position indicated by Pocock (1938), namely to *V. tertius*.

V. gigas from Kimberley, Africa, was placed in the section *Merrillosphaera* by Pocock (1933b).

In summary, therefore, the following revision of Printz' (1927) classification of the genus *Volvox* is suggested. 1. The inclusion of the following species in the section *Merrillosphaera* of the genus *Volvox: V. Carteri* Stein, *V. tertius* Meyer, *V. africanus* West, *V. gigas* Pocock. 2. The elimination of the section *Campbellosphaera*.

It was pointed out by Shaw (1919) in his description of *Campbellosphaera obversa* that this form of *Volvox* closely resembled *V. Carteri* and *V. tertius*. As indicated in Plate 1, the essential characters of *C. obversa* correspond very closely to those of *V. Carteri* except that *C. obversa* is homothallic whereas all forms of *V. Carteri* are heterothallic. After considering the above details the writer proposes that *Campbellosphaera obversa* be assigned to *Volvox Carteri* as *Volvox Carteri* var. **homothallicus** Metzner, var. nov. [= *Campbellosphaera obversa* Shaw; = *Volvox obversus* (Shaw) Printz].

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SUMMARY

1. A study of *Volvox Carteri* var. *Hazeni*, a new variety, is presented, with emphasis upon studies of the living organism.

2. Cultures were maintained in the laboratory during the summer months in solutions to which regular additions of iron salts were made.

3. The membrane and vegetative cell structure closely resemble those of *V. tertius* Meyer. Vegetative cells of crushed coenobia may become detached and free-swimming.

4. Asexual reproduction is characteristically by production of eight daughter colonies in which the reproductive cells are differentiated at about the 64-celled stage. No protoplasmic connections were visible in the development of daughter colonies, which undergo inversion in a manner similar to that described for other species of *Volvox*.

5. In sexual reproduction, *V. Carteri* is heterothallic. Female colonies produce 18–20 eggs. Male colonies are dwarf and contain about 50 bundles of spermatozoids. Fertilization was not observed nor was the presence of spermatozoids inside a female colony ever detected. It seems probable that a large number of eggs develop parthenogenetically. The oospore wall is reticulately ridged.

6. About 90 per cent germination was secured in oospore culture experiments. It was possible to obtain germination of oospores within 10 days after their release from a female colony. Germination of the oospore results in the formation of an aplanospore or biflagellate zoospore. Either product, aplanospore or zoospore, undergoes successive divisions to form a young colony which inverts to form a juvenile colony which is distinguished by its small size, few gonidia, and brownish color.

7. Two periods of maximum mitotic activity, one at about 3 p.m. and the other at approximately 10 p.m., were observed; but mitotic figures were not abundant.

8. The cells of developing daughter colonies divide synchronously.

9. Mitotic division is of the type generally occurring among the protista. A resting nucleus of a cell in a developing daughter colony contains a large Feulgen-positive endosome, a smaller Feulgen-negative plasmosome, and a linin reticulum with chromatic granules scattered through it. In prophase the plasmosome disappears and endosomal division results in the formation of two polar endosomal masses which undergo chemical changes while being extruded through the nuclear membrane into the cytoplasm. Intranuclear endosomal masses are Feulgen-positive whereas after extrusion they are Feulgen-negative. It is suggested that there is a flow of nucleic acids from nucleus to cytoplasm wherein the desoxyribose nucleic acid derived from the endosome becomes converted to cytoplasmic ribonucleic acid. Division of the endosome may be determined by the position of that body with respect to the spindle mechanism; division may be autonomous or finally it may be due to a combination of both of these factors. If the endosome lies in the spindle region, as is usually the case, it is divided into two polar masses; however, if the endosome is to one side of the spindle it is not divided, but gradually undergoes dissolution inside the nucleus.

10. The endosome may occasionally give off buds of pale-staining substance.

11. In metaphase the chromosomes are tiny and closely grouped and the endosomal masses have almost completely disappeared. The anaphases occur quickly and in telophase aggregation of chromatic material results in the reformation of the endosome; in addition the plasmosome reappears.

12. Mitosis is intranuclear. Cytokinesis is achieved by constriction of the protoplast between the daughter nuclei.

13. The evidence suggests that most of the oospores of *V. Carteri* var. *Hazeni* are parthenospores, although the possibility that fertilization occurs is not definitely excluded.

14. A revision of the section *Merrillosphaera* of the genus *Volvox* is suggested in which the species *V. mononae* Smith is abandoned and *V. Migulae* (Shaw) Printz is incorporated within another species. It is also proposed that the section *Campbellosphaera* be abandoned and the form *Campbellosphaera obversa* be named *Volvox Carteri* var. *homothallicus*.

15. A detailed description of *V. Carteri* var. *Hazeni*, var. nov., is presented together with pertinent data on the other members of the section *Merrillosphaera*.

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THE ANATOMY OF PEACH AND CHERRY PHLOEM[1]

HENRY SCHNEIDER[2, 3]

INTRODUCTION

In the course of a study of the effect of the buckskin virus upon the anatomy of peach (*Prunus persica* Sieb. & Zucc.) and cherry (*P. avium* L.), little literature was found concerning the phloem of peach. The development of the primary and secondary phloem in the healthy peach was therefore studied. Some attention was also given to the secondary phloem of healthy cherry. The results of these studies are herein reported. The literature on the anatomy of the peach is discussed at appropriate places in the text.

MATERIALS AND METHODS

Much of the material used was taken from plants grown in five-gallon cans or pots in the greenhouse. Other material was supplied by Dr. T. E. Rawlins from an experimental plot in Green Valley, Solano County, California. Part of the peach material was supplied by Dr. L. C. Cochran from his plots in Moreno Valley, Riverside County, California. For the most part the study was conducted on Orange Cling peaches on peach stock and Napoleon cherries on either Mazzard or Mahaleb stock.

To make a developmental study of the phloem of peach it was necessary to have sections of successively older parts of the stem beginning with the apical meristem. Serial cross sections of the stem tip were made to a point where elongation started. Below this point pieces were taken from the center of each successive internode and from the petioles of leaves at each node. In this way developmental stages of the shoot were obtained. Sections were also made of the phloem of older stems and of the trunk.

Only secondary growth of cherry stems was considered. The bark was taken from trunks and branches of various ages.

Three fixatives were used. Alcohol-formalin-acetic no. 1 (Rawlins 1933) caused some shrinkage but had the advantage of penetrating well, and its

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use was essential when the material was in large pieces. Karpechenkos's fixative caused some shrinkage in the cambial region but was quite satisfactory for other tissues. The least shrinkage occurred when Nemec's solution was used, but it had the disadvantage of fixing tannin-containing globules, which made photography difficult. Nemec's solution required a longer time for fixation. Cell walls stained very well, even near the apical meristem, with dilute Heidenhain's haematoxylin after the use of this fixative. In preparation for embedding in paraffin, the material was treated in a manner similar to that used by Ball (1941). However, a mixture of equal parts of cedar oil and xylene were substituted for xylene. Material embedded in celloidin was handled according to the method of Jeffrey as described by Chamberlain (1932).

Haupt's adhesive (Chamberlain 1932) was used when sections were to be stained, but Ullrich's adhesive (Johansen 1940) was found to be more satisfactory when sections were to be treated with neutral or strong acid reagents in microchemical tests. The latter adhesive is soluble in bases and does not hold sections on the slide when basic reagents are used. A number of stains were used. The method of Zimmerman (1922) was found to be satisfactory for temporary mounts. Varying proportions of aqueous solutions of 0.01 per cent azure II and 0. 01 per cent basic fuchsin were mixed just before using, and the sections were treated until the desired intensity was attained. The sections were then mounted in glycerol. Serial sections of stem tips and part of the other material were mordanted with iron alum and then stained in dilute Heidenhain's haematoxylin until the walls were sufficiently dark. Counter-staining with orange G in clove oil was used to impart a yellow color to the nacré walls of the sieve tubes and callus. Delafield's haematoxylin and safranin were used for staining some of the celloidin sections.

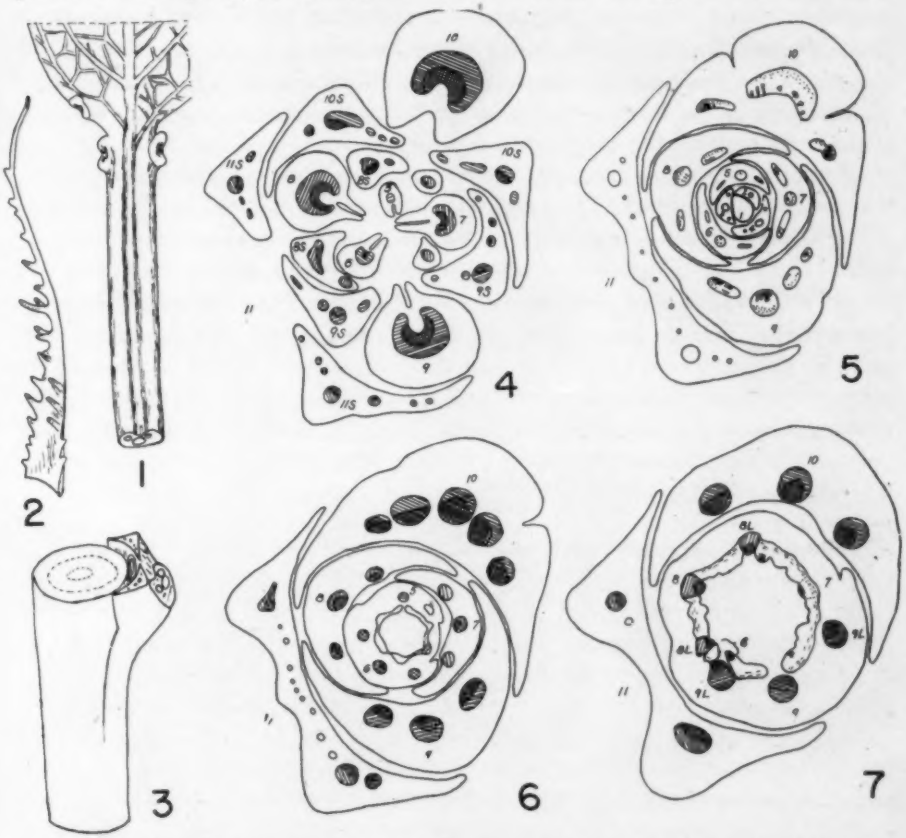
DEVELOPMENTAL ANATOMY OF THE PEACH PHLOEM

General Features of the Structure and Development of the Leaf. The leaf base partially sheaths the stem (fig. 3). The petiole of the leaf (fig. 1) is attached to the abaxial side of the leaf base in a median position. The stipules (fig. 2) are attached to the top of the leaf base.

Fitzpatrick (1934) studied the ontogeny of the peach leaf in connection with the peach-leaf-curl disease. The peach leaf appears first as a slight protuberance on the side of the apical meristem. The stipules later appear as subsidiary protuberances on both sides of this primordium. As the leaf primordium enlarges two lateral meristematic ridges appear on its adaxial face and finally develop into the leaf blade (fig. 4, leaves numbered 5, 6, 7).

With regard to the development of the peach leaf, Fitzpatrick observed the following: The glands which occur along the margins of the leaf appear and mature first at the tips of the leaves; the lateral veins differentiate first

at the apex, and the intercostal growth between the lateral veins is initiated here also; stomata first appear near the tip, and the differentiation into palisade and spongy parenchyma occurs first at the tip of the leaf, then



FIGS. 1–3. Stem, stipule, and petiole of peach. FIG. 3. Portion of a stem with a leaf base from which the stipules (fig. 2) and the petiole (fig. 1) were removed at the abscission regions. FIGS. 4–7. Cross section at several levels through a stem tip of peach. FIG. 4. 150 microns above the stem tip. Stipules appear near each side of the leaf primordia (the letter *s* indicates a stipule). FIG. 5. 50 microns below the stem tip. Dots represent sieve tubes and circles the lignified xylem elements. FIG. 6. 110 microns below the stem tip. FIG. 7. 375 microns below the stem tip. In all figures numbers represent the order of formation of the leaves, the leaf numbered 1 being the youngest. Cross hatching represents primary xylem and single hatching represents phloem. The letter *L* indicates a lateral trace. × 60.

at the base. In other words, the peach leaf, like many other leaves, matures basipetally.

Beneath the emerging leaf primordia, groups of cells within the stem elongate in a direction parallel to the axis and thus form the procambial

strands. The cells surrounding these areas enlarge isodiametrically to form the ground meristem. The ground-meristem cells have conspicuous vacuoles and therefore their protoplasts stain less densely than the cells of the procambial strands. In an actively growing stem, the second leaf primordium had a median strand, and the third primordium had a median and two lateral strands in procambial state (fig. 5, leaves numbered 2, 3). The youngest leaf primordium at the apical cone is referred to as number one and others are numbered according to their relative ages.

Each leaf has three traces and within the stem the traces fork and anastomose with each other and form together a network in the shape of a cylinder (dictyostele). The traces of a given leaf become recognizable some distance below the node at which they diverge into a leaf (fig. 7, traces of leaf 8). The three bundles that diverged into the leaf remain unchanged throughout the lower part of the leaf base. In the upper part of the leaf base the two lateral bundles are divided (fig. 6, leaf base 10). In the basal part of the petiole proper the inner branches of the two lateral bundles are united with the median bundle and this composite bundle continues through the rest of the petiole and the midrib (figs. 4, 5, leaf base 10). The outer branches of the laterals are stipular bundles.

Nomenclature Used and Characteristics of the Sieve Tubes. The nomenclature outlined by Esau (1938, 1939) for the description of the different stages of development of the phloem is followed in this paper. The part of the primary phloem which matures before the stem has ceased to elongate is termed the *protophloem*. In peach the sieve tubes of the protophloem, as seen in cross section, were of approximately the same diameter as the adjacent parenchyma and procambial cells (fig. 8). In paraffin sections the walls of protophloem sieve tubes were slightly thicker than those of the adjacent parenchyma cells. The mature sieve tubes had only thin parietal cytoplasm and appeared to lack nuclei. Companion cells, if present, were indistinguishable from other parenchyma cells. Sieve plates were observed in the protophloem sieve tubes.

The primary phloem which matures after stem elongation ceases is termed *metaphloem*. The sieve tubes of the metaphloem in peach were found to be larger than the adjacent parenchyma cells and showed very thick crenulated walls with a pearly luster. This type of sieve-tube wall has been described as "nacré" in the literature (Lesage 1891, as cited by Esau 1939). The nacré walls stained more lightly than the original procambial walls and the parenchyma-cell walls with Heidenhain's haematoxylin (figs. 15, 16). Companion cells were associated with the metaphloem sieve tubes. No sharp line of demarcation between the metaphloem and the protophloem was observed.

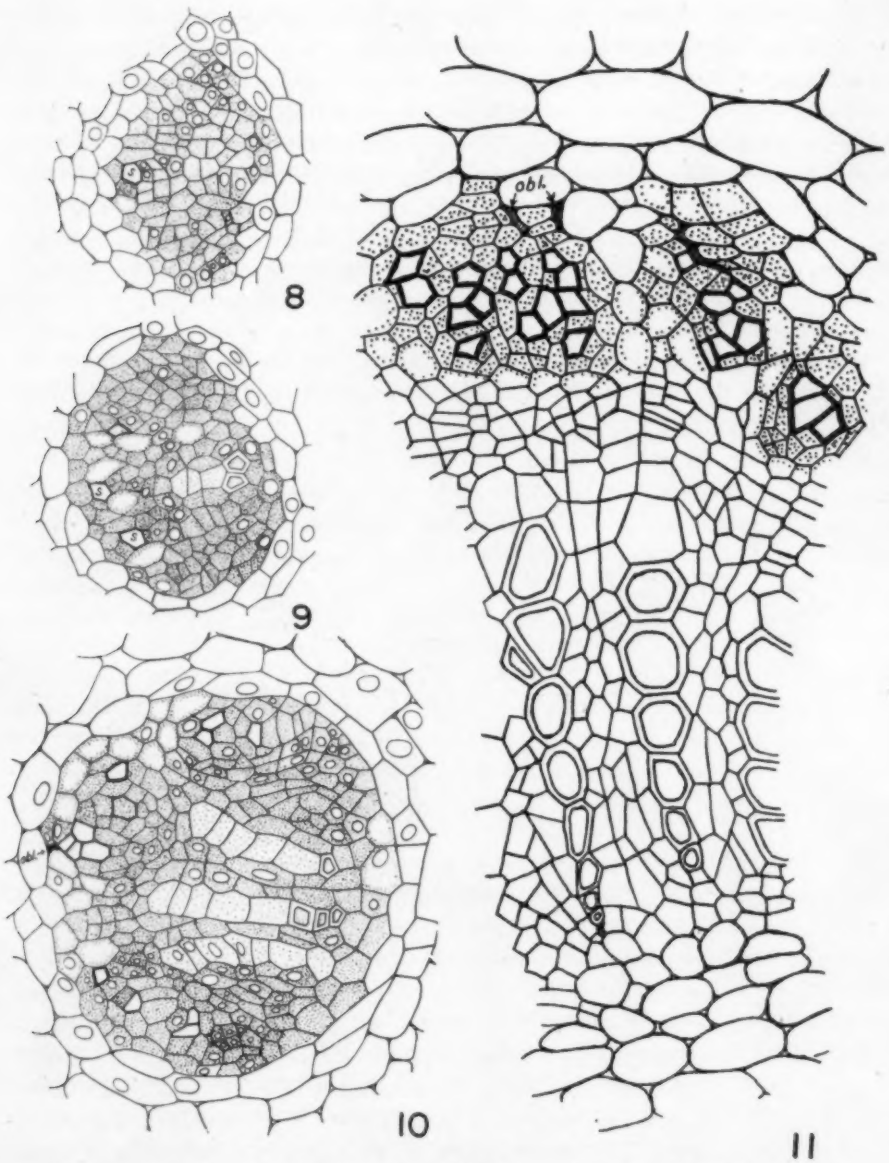
The phloem tissues initiated from the cambium are termed *secondary phloem*. As seen in cross sections of peach stems, the procambium is composed of cells polygonal in shape and periclinal divisions predominate in it. The procambium is confined to the primary bundles (fig. 12); whereas the cambium, except in early stages of transformation from procambium to cambium, forms a continuous cylinder of cells. The cambium cells are rectangular and divide in an orderly manner by periclinal walls during the formation of xylem and phloem. As seen in tangential section the cambial cells which initiate the vascular rays are about twice as long as wide and the initials which give rise to the longitudinal system are long fusiform cells.

Ontogeny of the Phloem Tissue of the Leaves. The study of the development of the phloem was begun by finding the youngest leaf base which

TABLE 1. *Relative age of the leaf primordium and differentiation of the first vascular elements.*

Stem tip number	The number of the leaf with the first				Nature of growth of stem
	Sieve tubes in the leaf base	Thick-walled xylem element in the leaf base	Phloem obliteration in the leaf base	Thick-walled xylem element in the petiole	
1	5	6	8	5	Rapid
2	5	8	.....	.....	Slowing down
3	5	5	7	5	Rapid
4	5	6	9	5	Rapid
5	6	8	8	8	Growth stopped

had a sieve tube in the median trace. Then the phloem of median bundles of successively older leaf bases was examined to determine where the next sieve tubes were formed in relation to the first and what the destiny of the first-formed sieve tubes was. The smallest leaf primordium at the apical cone is referred to as leaf number one, and the others are numbered consecutively according to their relative ages. A section 50 microns below the stem tip of a rapidly growing shoot showed that the median bundle of leaf base number 5 was the first to have a sieve tube (figs. 5, 8). This seems to be typical (table 1). The first sieve tube is initiated in the center of the abaxial margin of the bundle. No xylem elements were present at this level; however, one was present in the petiole region above. Leaf base number 6 had three sieve tubes, two additional sieve tubes having differentiated tangentially on either side of the first sieve tube (figs. 5, 9). Two lignified immature xylem elements had developed in the center of the adaxial side of the bundle. Adjacent vacuolated cells on the abaxial side of these elements were arranged in radial rows as though they were initiated from meristematic cells by periclinal divisions



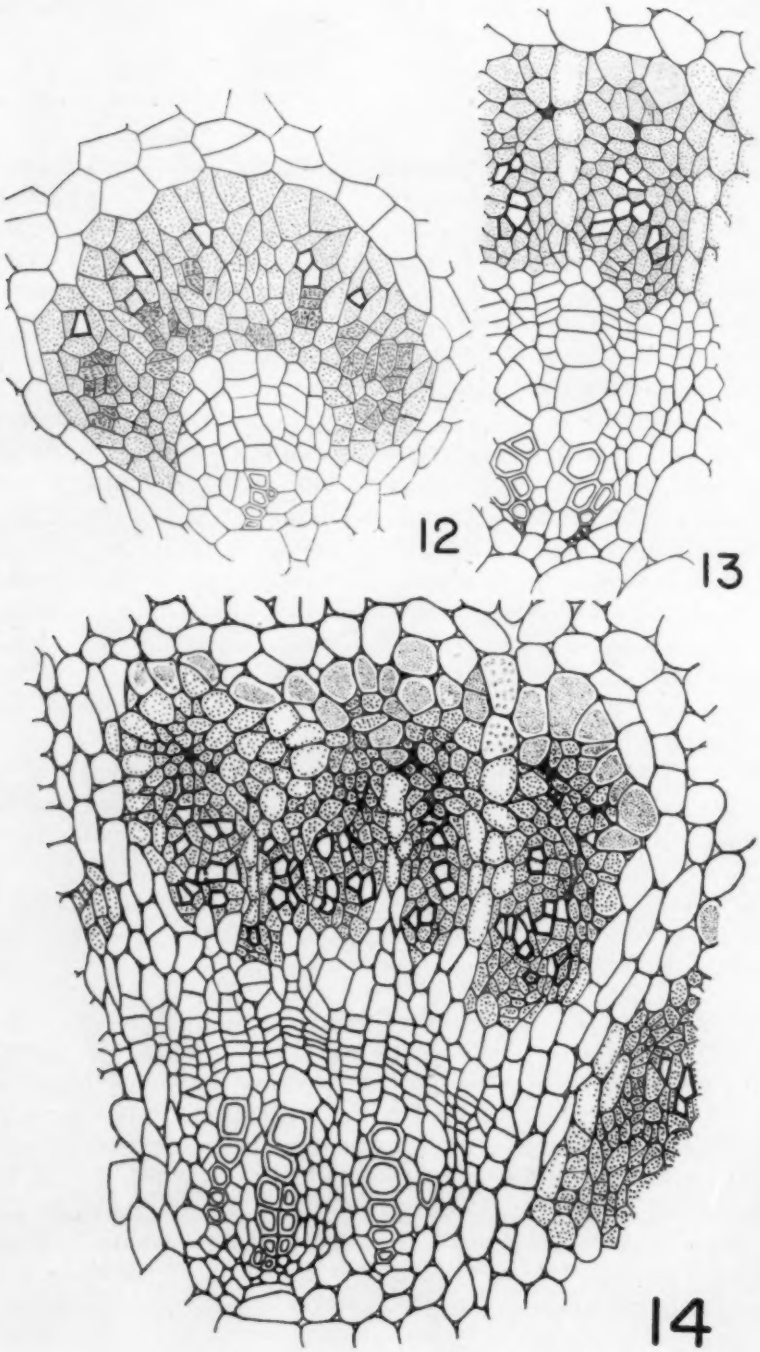
Cross sections though median bundles of leaf bases of peach. FIG. 8. From leaf base number 5. FIG. 9. From leaf base number 6. Figures 8 and 9 are 50 microns below the stem tip. FIG. 10. Bundle from leaf base number 8, 110 microns below the stem tip. FIG. 11. Portion of a bundle from leaf base number 14, 6 mm. from the stem tip. Stippled cells indicate the procambium, primary xylem and primary phloem in figures 8, 9, and 10, but only the primary phloem in figure 11. Areas inked solid or single hatched areas between cells represent obliterated phloem (*obl.*). Stippled cells with thick walls represent immature sieve tubes. Thick walled cells not stippled represent mature sieve tubes (*s*). All × 650.

(fig. 9). They were vessel initials. At this stage the phloem region also showed a tendency toward radial arrangement of cells.

The first sieve tube was found to be obliterated in the seventh to ninth leaf bases (table 1). In the median bundle of leaf base number 8 of one stem studied, obliteration of the first sieve tube had occurred (fig. 10). New sieve tubes had differentiated along the abaxial margin of the trace and centripetal to them. In this trace the phloem was becoming divided into strands by enlargement of rows of parenchyma cells which are the beginnings of the primary rays. In the same stem in the median bundle of leaf base number 14, (fig. 11), 6 mm. from the stem tip, several sieve tubes were obliterated and new ones had arisen in a centripetal direction. The phloem region had been definitely divided into separate strands by the primary rays.

The metaphloem remains in a functioning state throughout the life of the leaf. In the differentiation of the petiole the last cells of the procambium to be initiated on the phloem side develop into large parenchyma cells which separate the primary and secondary phloem (Schneider 1945, photomicrographs of petiole sections). Only a small amount of secondary phloem is produced in the petiole and midrib of leaves. As seen in a radial section in an old leaf, the cells of the cortex which border the protophloem bundles vary from isodiametric to rectangular. The protophloem cells which remain intact after the obliteration of the sieve tubes become much elongated (1–1.5 mm. in length) and increase in cross-sectional diameter. Similar cells in the stem lay down secondary walls which become lignified. The metaphloem contains sieve tubes, companion cells and phloem parenchyma cells. In radial section the parenchyma cells are variously shaped elongated cells and the sieve tube elements are also elongated and variously curved. The parenchyma cells separating the primary and secondary phloem are from cubical to isodiametric. The phloem tissue of the lateral veins is similar to that of the midrib and petiole except that only a few strands of primary phloem are present and no secondary phloem is produced. The protophloem is obliterated in the same manner as in the petiole.

Ontogeny of the Phloem in the Stem. In early stages the traces of the stem develop similarly to those of the leaf. The first sieve tube appears just inside a row of cells that constitute the outermost portion of the procambial strand. The next sieve tubes develop in a tangential direction on both sides of this first sieve tube (fig. 12). The first sieve tubes are then obliterated as new ones differentiate in centripetal direction (figs. 13, 14). At this stage of development primary rays divide the primary phloem into strands (figs. 13, 14). When branching of traces occurs at certain levels of the stem, the primary rays become wider to form the leaf gaps. This occurs above the point where other leaf bundles enter the vascular cylinder. As the protophloem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sieve tubes are obliterated fibers differentiate (fig. 15). The origin of fibers is described below. Centrally to the fibers there is a region of sieve-tube obliteration and then a region of metaphloem.

Fibers of the primary bundles arise from procambial cells which did not differentiate into sieve tubes. These procambial cells elongate, increase in diameter, lay down secondary walls, and finally become lignified. Because of their procambial origin these fibers are here termed primary-phloem fibers. Until recently (Esau 1938, 1943), primary-phloem fibers have been generally interpreted as pericyclic fibers (Eames & McDaniels 1925; Hayward 1938). The fibers of the outer row have different characteristics from the other primary fibers throughout their development; they enlarge first (fig. 12), their lumina are filled with a heavily staining substance (fig. 14), they become larger and are lignified later than the subjacent fibers (fig. 15).

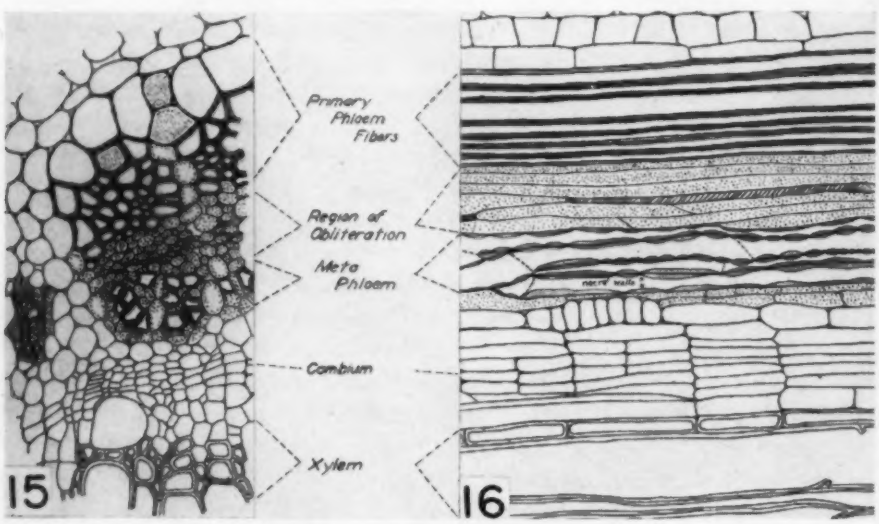
The strands of primary phloem eventually become bordered on their inner sides by large parenchyma cells initiated by the procambium after the full complement of the primary phloem mother cells are formed. Primary xylem initials are laid down more slowly than initials of the phloem until the full complement of primary phloem cells is formed. (Compare the median trace of leaf number 7 in fig. 12 with that of leaf number 9 in fig. 13.) The strands become elliptical in shape because of the tendency of the phloem mother cells to divide periclinally with respect to strands; and because of the manner in which the primary rays broaden out (fig. 14).

Partly developed primary-phloem strands are distinctly isolated from each other; and as seen in transverse sections, they appear to be meristematic on their inner sides because of the presence of small cells which contain dense protoplasm. A study of successively older traces explains how these strands are isolated and why they appear to be meristematic on their inner sides. After having been added to the strands by divisions of the procambium, most of the new phloem cells (the phloem mother cells) divide again to form the young sieve tubes, companion cells, and phloem parenchyma cells. Because of these divisions the young phloem cells appear smaller than the

Explanation of figures 12–14

Transverse sections of leaf traces from the peach stem. FIG. 12. Median trace from leaf number 8, 240 microns from the stem tip (×800). Sparsely stippled cells represent primary phloem and procambium cells. Cells with dashes represent those that arose from the most recent divisions. Those nearest the unstippled xylem area belong to the procambium and others are recently divided phloem-mother cells. FIG. 13. Median trace from leaf number 9, 2400 microns from the stem tip (×400). Figures 12 and 13 are drawn from sections from the same stems as the drawings in figures 4–11. These sections were taken from a rapidly growing stem. FIG. 14. A lateral trace of leaf 11 about 700 microns from the tip of a stem which was growing slowly (×400). In figures 13 and 14 stippled cells belong to the phloem strands. Finely stippled cells contained copious precipitates, and represent immature primary-phloem fibers. Thick-walled cells represent sieve tubes. Single hatched areas represent obliterated sieve tubes.

procambial cells from which they were derived. The sieve tubes of this part of the phloem mature slowly and increase in length before they increase in diameter. Because of the slow maturation this part of the phloem remains meristematic in appearance for a considerable time (fig. 14). While the inner sides of strands are maturing into the metaphloem, the initiation of xylem becomes more active and the procambium is transformed into the cambium, which at first only gives rise to xylem (figs. 14, 15). After a considerable



Sections from peach stems. FIG. 15. Transverse section of part of the primary phloem of the middle trace of leaf number 13, just below the level where it anastomoses with the vascular cylinder, and 7.5 mm. from the stem tip. FIG. 16. A bundle of about the same age in radial longitudinal section. Both × 380. In both figures sparsely stippled cells represent phloem parenchyma and companion cells. Single hatched areas between cells represent crushed sieve tubes and companion cells. Cells with shaded crenulated walls are sieve tube elements. Cells with thick shaded secondary walls which are not crenulated are primary phloem fibers. Densely stippled cells represent immature primary phloem fibers with vacuolar precipitates. Other large cells in figure 15 which border the outer side of the fibers are immature primary-phloem fiber initials from which the heavy precipitates were lost in preparing the sections. This outer row of fibers lays down secondary walls later than those just centrally to it.

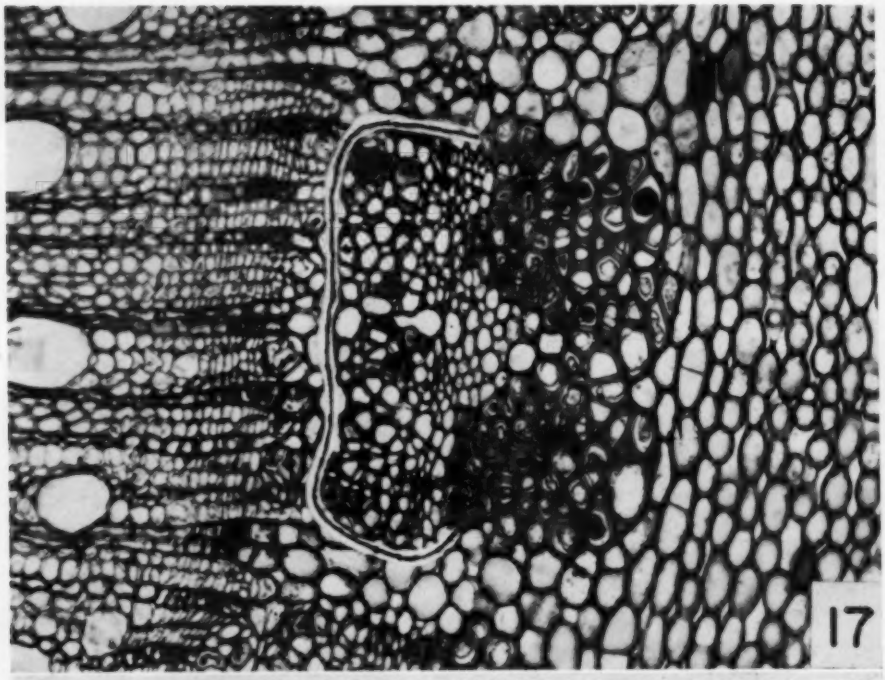
amount of xylem has been produced, the secondary phloem begins to be formed (fig. 17).

In a growing stem 32 cm. from the stem tip, secondary phloem was being produced. The secondary sieve tubes had thick nacré walls, and the nacré walls of some of the metaphloem sieve tubes had disappeared (fig. 17). A row of parenchyma cells (the origin of which was previously described) separated the primary phloem from the secondary phloem (fig. 17). Eames and McDaniels (1925, p. 107) picture a magnolia stem in which the primary and

secondary phloem are similarly separated. At certain levels the secondary and primary phloem anastomosed with each other. In the same stem 85 cm. from the stem tip, more secondary phloem had been produced and most of the sieve tubes of the primary phloem were obliterated (fig. 18). The nacré walls of the secondary sieve tubes stood out very distinctly. The walls of the primary-phloem fibers had thickened and were difficult to cut.

When a peach shoot ceases to grow, the apical cone becomes obtuse, and is sheathed by large fleshy leaf bases. A leaf trace within the stem from any given leaf may be in various stages of development depending on the nature of the growth of the stem. The median traces from leaf number 13 of two rapidly growing stems were observed to have their full complement of primary phloem cells and secondary walls were just beginning to be laid down in the primary-phloem fibers. In the primary phloem of the median trace of leaf number 13 of a stem in which growth had nearly stopped, the full complement of cells was present, and the secondary walls of the primary-phloem fibers were almost completely laid down (fig. 15). In a stem which had not produced apical growth for several weeks the fibers were completely lignified in the median trace of leaf number 13, much of the metaphloem was still functioning, and some active secondary phloem was present. The phloem of traces of younger leaf primordia remained at approximately the same stages of development in all types of shoots (table 1, stem tip 5). In a stem tip of Hale peach from Southern California which had ceased to elongate there were just a few isolated fibers which were completely lignified in the median trace from the thirteenth leaf. The walls of other fiber initials were just beginning to lignify and secondary sieve tubes were present.

Anatomy of the Peach Stem Undergoing Secondary Growth. A peach stem in the early stages of secondary growth consists of the following tissues as observed in transverse sections. The outermost tissue of the stem is composed of a single layer of epidermal cells which has a thick cuticle. Inside this there are elliptical parenchyma cells which are arranged in periclinal rows, with their long axes oriented tangentially (fig. 19). This is the only type of cell in the cortex. Occasional cortical cells may contain calcium oxalate crystals which fill the cells and cause them to enlarge greatly, and in some instances to disintegrate (fig. 19). Large air spaces occur between the tangential rows of cortical cells. As the stem becomes less succulent, a periderm is formed. As De Bary (1884) reported, it is formed in the layer of cells immediately below the epidermis. Eames and McDaniels (1925) record a seasonal activity of a persistent cork cambium in *Prunus serotina*. This is also the case in peach. Next to the cortex occur the isolated groups of primary-phloem fibers. Inside these occur tangentially flattened parenchyma cells and sieve tubes of the primary phloem (fig. 19). As the stem increases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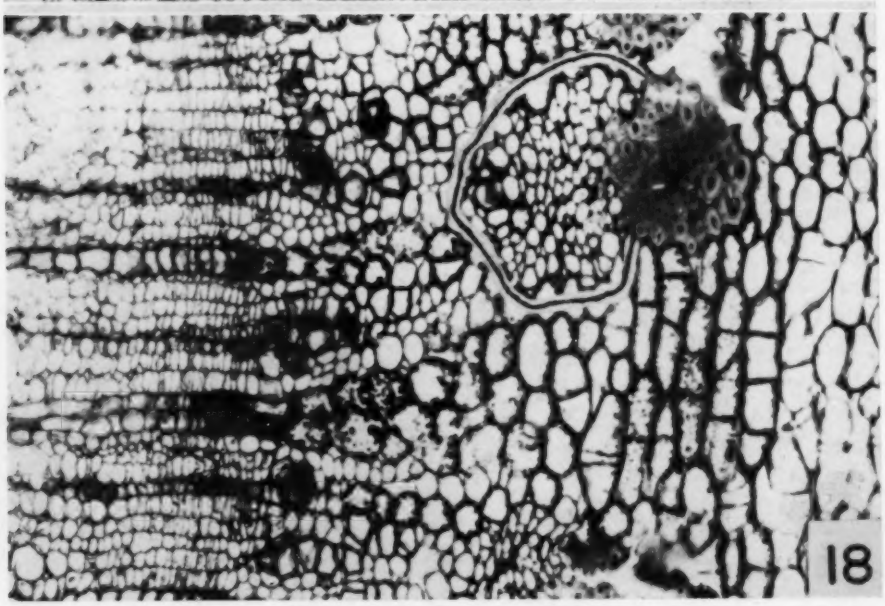
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in circumference, the primary phloem bundles (consisting of primary-phloem fibers and inactive metaphloem) become separated. Increase in girth is accompanied by tangential elongation and division of the parenchyma cells between them (fig. 18). A ring of secondary phloem occurs between the primary phloem bundles and the cambium. It is divided into blocks by radially arranged vascular rays. The blocks of secondary phloem contain thin-walled parenchyma cells, thick-walled sieve tubes which have transverse or oblique sieve plates, and companion cells. Some phloem parenchyma cells may contain calcium oxalate crystals which cause the cell walls to distintegrate. De Bary (1884) noted similar cavities in *Prunus*. Centrally to the phloem lies the vascular cambium, then follows the xylem.

In stems two or more years old, secondary growth causes the previous year's phloem to be crushed against the primary-phloem fibers. Sometimes the phloem ray cells are also crushed, but more often they are bent by the increase in girth. Cells of ray portions several layers thick may be elongated tangentially and the girth of the obliterated phloem region thus increased. When this occurs the length of such rays in the crushed phloem region is decreased without bending of the rays. The primary-phloem fibers become isolated in tissue composed of tangentially elongated parenchyma cells formed from the folded vascular rays. Thin layers of flattened cells may be seen curving into this parenchymatous tissue, each of which represents a crushed block of secondary phloem. Because of the obliteration of the non-functioning phloem, the bark of peach trees remains relatively thin.

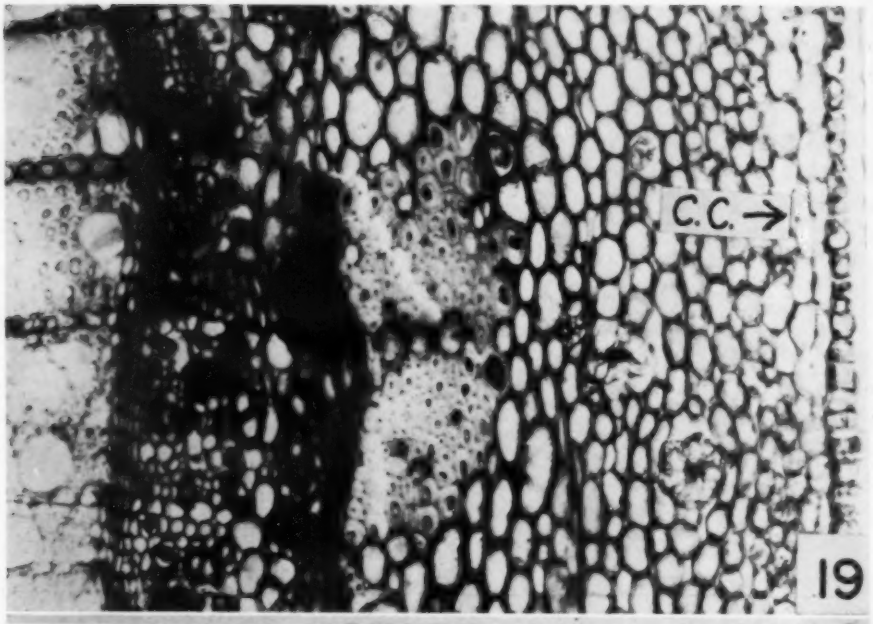
ANATOMY OF SWEET CHERRY BARK

Bastin (1895) made a study of the general features of bark several years old as it appeared in some of the cherries native to North America. The species considered were *P. serotina* Ehphart, *P. avium* L., *P. mahaleb* L., *P. pennsylvanica* L., and *P. virginiana* L. His terminology is difficult to understand and it seems desirable to redescribe the anatomy of the bark of *Prunus avium* from the point of view of ontogeny and seasonal changes.

Young sweet cherry stems in the process of secondary growth are very much like those of peach. The cells of the cortex are arranged in tangential rows with air spaces between the rows. Sclereids (which are absent in the peach) eventually ramify through the cortex. The development of these

Explanation of figures 17, 18

Transverse sections from peach stems. FIG. 17. 32 cm. from the stem tip and in the 6th fully elongated internode. Secondary phloem is just beginning to form. × 230. FIG. 18. 85 cm. from the tip of the same stem. Most of the sieve tubes of the primary phloem have been obliterated. Sieve tubes of the secondary phloem have thick crenulated walls. Lumina of some of the sieve tubes in the secondary phloem appear closed because of the presence of sieve plates. × 180. One primary phloem bundle is set off by a line in each photograph. Celloidin sections stained with safranin and Delafields haematoxylin.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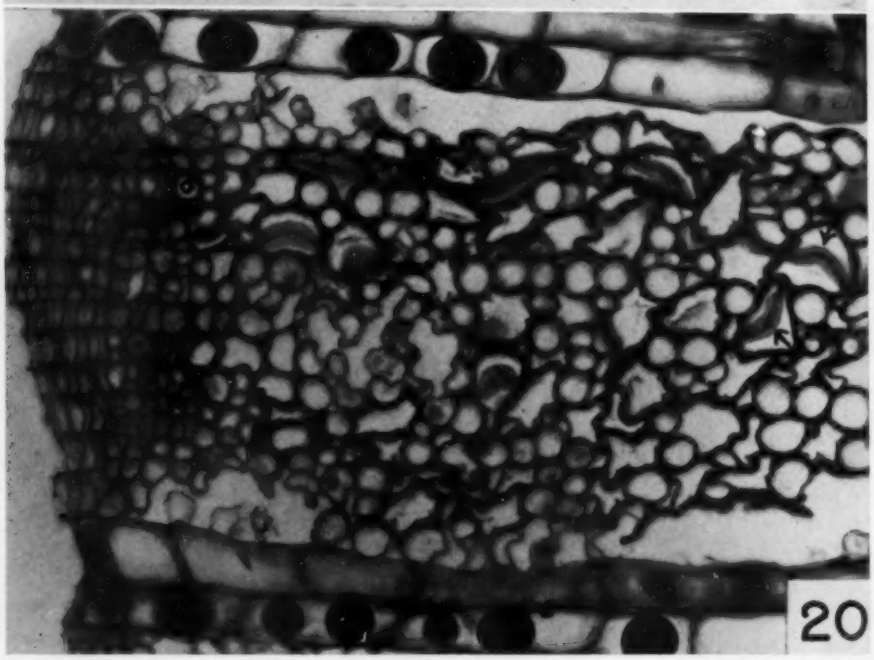
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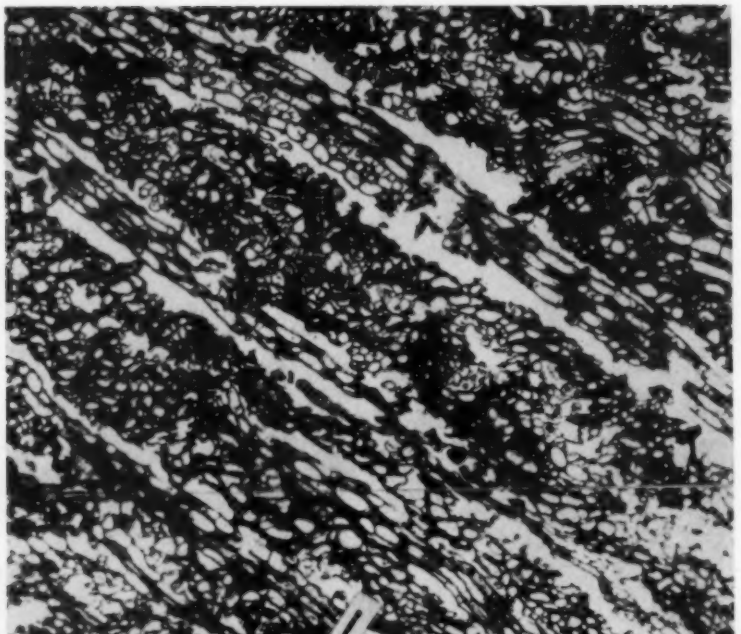
variously branched and shaped cells from parenchyma like initials has been described by Bergmann (1913) in *Prunus*. Primary-phloem fibers differentiate in the outer portion of the primary phloem bundles, and metaphloem is eventually crushed centrally to these. Primary phloem bundles are separated at most levels in the stem from the secondary phloem and from each other by large parenchyma cells. The secondary phloem area may be easily distinguished from the primary phloem by the straight rows of ray cells which it contains. No mature fibers are present in the functioning secondary phloem. They mature each growing season in the non-functioning phloem of the previous year (fig. 21).

In stems more than one year old, the phloem of previous years is not completely crushed by the new growth as it was found to be in peach. The partially crushed phloem is composed of rays several cells wide between which are cavities, parenchyma cells and partially collapsed sieve tubes. Secondary phloem fibers differentiate between the rays of the non-functioning phloem. They are not in bundles as are the primary phloem fibers but intermingle with old conductive tissues (fig. 21). Occasional sclereids of the type found in the cortex are present. The rays in phloem several years old appear wavy in transection, and the longer ones terminate at the primary phloem fibers.

In the fall and winter the layer of phloem present contains sieve tubes and parenchyma cells which are smaller than those found in the summer. These sieve tubes were without definitive callus on the sieve plates on January 5, 1942 (fig. 20). Summer sieve tubes were partially collapsed and definitive callus was present on the sieve plates. Parenchyma cells located near the rays and containing calcium oxalate crystals deteriorate and thus cause the formation of the cavities between the rays and the phloem tissue. During the summer fibers mature in the phloem of the preceding season. They are more abundant in the phloem of the previous autumn and winter than in the previous year's summer phloem (fig. 21). Gill (1932) has found in ash (*Fraxinus excelsior* L.) that large summer sieve tubes and small autumn sieve tubes are produced. Elliott (1935) on the other had found in *Acer pseudoplatanus* L. that large spring sieve tubes differentiate from thin-walled cells present during the previous winter and that the sieve tubes produced continuously during the summer from renewed action of the cam-

Explanation of figures 19, 20

FIG. 19. Transverse section of the bark of peach (Hale variety). Some subepidermal cells have divided to initiate the cork cambium (c.c.). Calcium oxalate crystals have caused two cortical cells to enlarge and decompose. The primary phloem has been partially flattened against the primary phloem fibers. Sieve tube walls stained deeper than phloem paranchyma. × 200. FIG. 20. Transverse section of Sweet Cherry stem. Collected Jan. 25, 1942, at Green Valley. Summer sieve tubes are partially collapsed and sieve plates are callused (arrows point to 2 callused sieve plates). Cavities have developed between the rays and the phloem area. Thick-walled cells near the cambium with no contents are winter sieve tubes (two of them have circles drawn in their lumina). × 370.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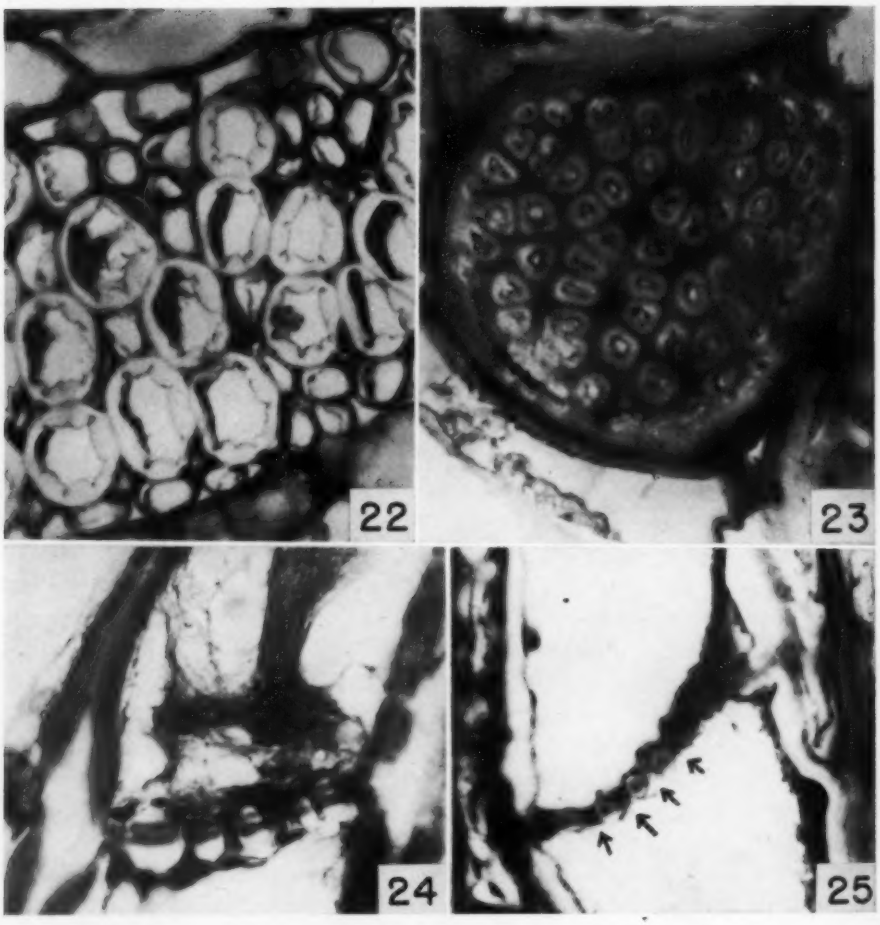
bium are relatively smaller. In sweet cherry the callus disappears from the partially collapsed sieve tubes the summer following their cessation of activity.

The summer sieve tubes are very large and may be the dominant type of cells between the rays (figs. 21, 22). The middle lamella and the original primary walls of sieve tubes contain pectic substances and stain black with Heidenhain's haematoxylin. The nacré walls which are reported to be of almost pure cellulose (Esau 1939) stain red with congo red, a cellulose stain (Conn 1940) (fig. 22). Slit-shaped cavities have been observed in some preparations in the nacré walls, and they are arranged in a concentric manner around the entire cell wall. In a longitudinal section of a sieve tube which was stained for cellulose with IKI and sulfuric acid (Rawlins 1933), the middle lamella and callus dissolved away, the cell walls were blue, and the protoplasmic connections turned yellow (fig. 24). Protoplasmic connections in some sieve plates appeared to be tubular when observed in transverse section (fig. 23). The material for sections in which this phenomenon was observed was collected in July at Green Valley and was fixed in alcohol-formalin-acetic no. 1 (Rawlins 1933), sectioned 2 microns thick in paraffin, stained by the azure II and basic fuchsin method, and mounted in glycerol. In material prepared in this manner (fig. 23) the black area between the protoplasmic connections is the cell wall, white rings are callus and the black rings inside the white rings are presumably protoplasm. In longitudinal view, protoplasmic connections also appear tubular (fig. 25). Connections also appear tubular after the same material is stained with safranin and gentian violet, eosin, or Heidenhain's haematoxylin. The tubular nature of the connections was only apparent in the large summer sieve tubes of vigorously growing orchard trees.

The nature of the connections between sieve tube elements still appears obscure. Some workers describe these connections as protoplasmic tubules enclosing vacuolar material (see review by Esau, 1939). Crafts (1932, 1933, 1939a, 1939b) and some other workers, however, doubted that the connections between sieve-tube elements are tubular and interpret them as solid protoplasmic strands. It is suggested here that slime which ordinarily coagulates on sieve plates during the process of removing and fixing stems for sectioning may plug the tubular connections and cause them to appear solid. Crafts (1933) and others have shown that sieve tube slime is suspended in

Explanation of figure 21

Transverse section of Sweet Cherry stem. Collected in July 1941. There is a wide band of functioning summer phloem next to the cambium. Large cells between the rays are sieve tubes and small cells are phloem parenchyma and companion cells. On the outside of the functioning phloem part of the obliterated phloem of previous years is shown. Sieve tubes and some of the parenchyma cells are partially or completely crushed. Fissures occur along the rays. The arrow points to fibers which have appeared in the degenerating tissues. × 88.



Sections of Sweet Cherry sieve tubes. Collected in July 1941. FIG. 22. Transverse section stained with Heidenhain's haematoxylin and Congo red. Mounted in glycerol. The original cambial walls and middle lamella stained black and the nacré walls stained red. × 575. FIG. 23. Sieve plate in face view and free of slime. Stained with azure II and basic fuchsin; mounted in glycerol; cut 2 μ thick. Cell walls and cytoplasm are black, callus rings are white. × 1500. FIG. 24. Longitudinal section of sieve tube at a sieve plate. Treated with IKI and mounted in 50 per cent sulfuric acid. The pectic middle lamella and the callus have dissolved. Protoplasmic connections are present. Slime is present on one side of the sieve plate and also fills the protoplasmic connections. × 1130. FIG. 25. Longitudinal sections of a sieve tube at a sieve plate. Stained with azure II and basic fuchsin and mounted in glycerol; sectioned 4 μ thick. Original cambium walls and cytoplasm are black. Callus, middle lamella, and nacré walls are white. Position of the vacuolar connections are shown by arrows. × 1125.

the vacuoles of the elements. When a segment of a stem is removed, sieve tube contents move from the center of the segment toward each end and are coagulated by ordinary fixatives on the side of the sieve plates away from the cut

surface. The sieve plates in the center of the stem segment remain free of slime since the contents of adjoining elements move in opposite directions (Crafts 1939a, fig. 5). Sieve plates of figures 23 and 25 are free of slime and consequently the connecting strands appear tubular. Figure 24 shows slime accumulated against one side of a sieve plate and here the strands appear solid.

SUMMARY

The development of the primary and secondary phloem of peach and the secondary phloem of cherry have been studied.

In the peach the first protophloem sieve tube was found in the fifth or sixth leaf primordium. The first sieve tube develops in the center of the abaxial side of the median leaf trace. Subsequent ones develop laterally and adaxially to the first. As seen in transverse section of a young leaf trace, the procambium is a layer of polygonal cells between the primary xylem and primary phloem which usually divide more nearly in a periclinal than an anticlinal direction. Certain cells initiated by the procambium differentiate into primary ray cells which divide the primary phloem into strands. As obliteration of protophloem sieve tubes occurs, the outer cells of the protophloem elongate, lay down secondary walls, and become lignified, thus forming the primary-phloem fibers. Elongation of similar cells without formation of secondary walls or lignification occurs in the petioles and leaves. There is a lag between the time at which the last metaphloem mother cells are initiated and that when the production of secondary phloem in the stems begins. During this lag, the last cells to be initiated on the phloem side by the procambium enlarge to form large parenchyma cells which separate the secondary phloem from the primary phloem, and metaphloem sieve tubes slowly develop from initials previously laid down. The procambium is then transformed into the cambium which initiates only xylem during the period in which the metaphloem sieve tubes differentiate from the mother cells previously laid down.

As the stem increases in girth by cambial activity, the strands of primary phloem fibers become removed from each other; they eventually appear on the periphery of the secondary phloem as isolated groups of fibers with crushed metaphloem on their inner sides.

For a time following maturation, secondary sieve tubes have thick nacré walls which nearly fill the lumina of the sieve tubes.

Secondary phloem of previous years is quite completely crushed and obliterated by newly produced phloem in peach. Hence the bark of peach remains thin.

In sweet cherry the new band of secondary phloem produced each spring functions during the summer and autumn. In late autumn the sieve tubes partially collapse and definitive callus forms on the sieve plates. A few small

sieve tubes near the cambium maintain their turgidity throughout the winter. During each growing season secondary phloem fibers mature in the phloem formed in the previous summer. Fissures occur along the rays in this old phloem tissue. Little crushing of old secondary phloem occurs in cherry and therefore the bark of cherry is thicker than that of peach.

Protoplasmic connections through the pores of sieve plates of sweet cherry are very large and appear to be tubular in sieve plates free of slime.

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FURTHER STUDIES ON THE ANTIBIOTIC ACTIVITY OF LICHENS*

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The production by bacteria, molds, algae, and flowering plants of antibiotic substances inimical to the life processes of other organisms has been clearly demonstrated with simple laboratory techniques by which small amounts of the active materials are supplied to assay microorganisms cultivated under suitable conditions. In an earlier report from our laboratory it was shown that lichens also possess antibacterial properties (1). Continuation of these studies has led to the accumulation of additional data concerning the antagonistic effects observed when approximately one hundred kinds of lichens were tested against selected species of bacteria including some pathogenic strains. We present herewith a summary report together with a brief discussion concerning the possible significance of the observations.

METHODS

The lichens were collected principally in Connecticut, in the Cape Cod region of Massachusetts, and in the White Mountains of New Hampshire. Some specimens were gathered for this work also by Dr. A. J. Riker in Wisconsin, and by Mr. C. A. Weatherby while on vacation in New Brunswick. Specimens from the Alcan Highway were supplied by Dr. H. M. Raup. Dr. Werner Bergmann also made available some interesting materials from the Sterling Laboratory of Chemistry of Yale University. The lichens were placed in shallow pans in the laboratory, moistened periodically with water, and exposed to daylight for the purpose of keeping them physiologically active. It should be mentioned, however, that a few of our later tests on dried specimens indicate that the active materials, in some lichens at least, are stable during storage for many years. Extracts were prepared by grinding with a glass mortar and pestle 100 mg. of lichen in 1 ml. of phosphate buffer solution adjusted to pH 7.4. The buffer solution was made by mixing 80.8 ml. of M/15 Na_2HPO_4 and 19.2 ml. of M/15 KH_2PO_4. The reaction of the aqueous suspensions of lichen materials was measured with color indicator papers before making the tests, and when necessary the pH was adjusted with dilute alkali to 7.0.

The assay procedure involved the use of the cylinder plate method of testing antibiotic potency of the extracts against bacteria in Petri-dish cultures (2). Agar plate suspensions of the test bacteria were prepared in the

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usual way and, after gelation of the medium was complete, small glass cylinders were arranged on the surface of the agar as indicated in figures 1 and 2. Difco yeast-beef-dextrose agar was employed as the nutrient medium, and the cups were "penicillometers" purchased from the Fisher Scientific Company. With a free-flowing pipette, 0.2 ml. of each lichen preparation was transferred to a cup. After a period of incubation, usually 15 hours at 37° C, zones of bacterial growth-inhibition subjacent to the cylinders were taken as an indication of antibacterial potency.

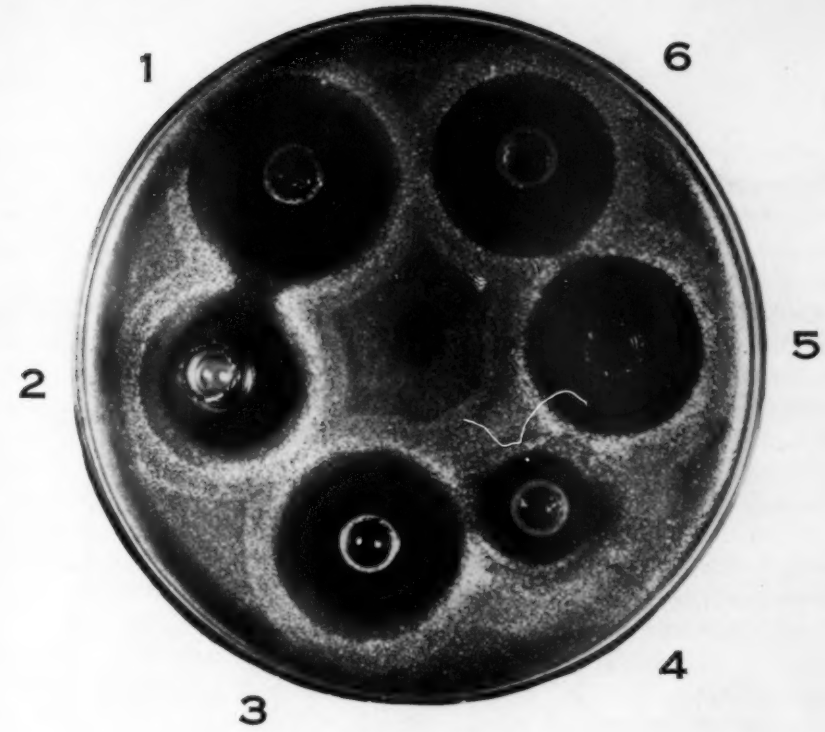
An attempt was made to test each of the collected lichens against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. In addition to these, certain other bacteria which were used in testing some of the lichens are the following: *Aerobacter aerogenes*, *Alcaligenes fecalis*, *Proteus vulgaris*, *Serratia marcescens*, *Bacillus cereus*, *B. mycoides*, *Clostridium Welchii*, *Diplococcus pneumoniae*, *Lactobacillus casei*, *Sarcina lutea*, *Staphylococcus albus*, *Streptococcus hemolyticus*, and *S. viridans*. Some of these organisms were supplied by Prof. S. A. Waksman, Rutgers University, and others were obtained through the kindness of Prof. P. B. Cowles, Yale University School of Medicine.

OBSERVATIONS

The results of our tests with different lichen extracts show varying degrees of activity ranging from strong growth promotion to marked inhibition. Some characteristic inhibition zones which were obtained with *B. subtilis* and *S. lutea* are illustrated in figure 1. Concentric patterns of growth promotion lying outside the region of inhibition, as in the plate of *B. subtilis* shown in the upper part of the figure, are found frequently in these tests and are the object of discussion in another paper (3). Tests with the sensitive *S. lutea* yield comparatively large zones of inhibition, as is shown in the lower part of figure 1. During the relatively long incubation period of about 36 hours necessitated by the slow growth of this organism, there is sufficient time for diffusion of an antibacterial substance to a considerable distance from the cup. The extent to which diffusion of the antibiotic substance takes place prior to growth of the test bacteria determines largely the diameter of the zone of inhibition. Both *Bacillus subtilis* and *Sarcina lutea* are excellent test organisms for use in teaching-demonstrations of antibiosis, provided smooth suspensions of the bacteria are employed in making the pour plates. Relative stability of at least some of the antibiotic lichen compounds is indi-

Explanation of figure 1

Antibacterial action of lichen extracts at pH 7.0 in cups, tested against bacteria in yeast-beef-dextrose agar. Above, *B. subtilis* grown for 16 hours at 37° C; 1, *Cladonia pleurota*; 2, *C. mitis*; 3, *C. uncialis*; 4, *Stereocaulon paschale*; 5, *Parmelia caperata*; 6, *Cladonia sylvatica*. Below, *S. lutea* grown for 36 hours at 25° C; 7, *Cladonia Grayi*; 8, *C. sylvatica*; 9, *C. cristatella*; 10, *C. rangiferina*.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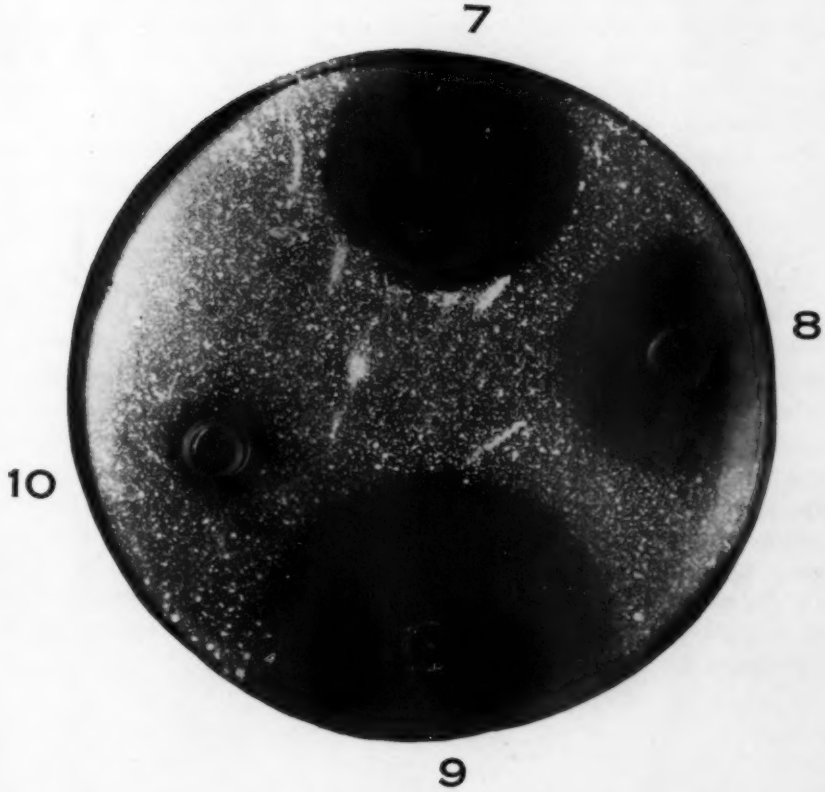
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cated by the fact that the tests with *Sarcina lutea* shown in figure 1 were made with lichen materials which had been allowed to dry in the laboratory for several months. The activity of several species of lichens was not lost by boiling in Na_2CO_3 solution for several minutes, which indicates that we are dealing with substances very different from penicillin.

It has been found generally that samples of a species of lichen collected from different regions show characteristic activity in antibiotic tests with suitable bacteria. Occasionally, however, some samples of a species which hitherto has given indications of activity fail to inhibit bacterial growth. No satisfactory explanation for this variability is known, although it may be of significance to point out that some of the diagnostic acids have been reported to occur in varying amounts in different samples of some species of lichens.

Thirty-five species of *Cladonia* have been found active against either *B. subtilis* or *S. aureus* or against both of these bacteria. Species observed to be active against both test bacteria are the following: *C. borbonica, C. caespiticia, C. capitata, C. cristatella, C. glauca, C. Grayi, C. merochlorophaea, C. nemoxyna, C. papillaria, C. pyxidata* (variable against *B. subtilis*), *C. squamosa,* and *C. strepsilis* (somewhat variable for *B. subtilis,* see figure 2, no. 4). Species inhibiting *B. subtilis* but not *S. aureus* are: *C. amaurocraea, C. alpestris, C. atlantica, C. bacillaris, C. Boryi, C. brevis, C. caroliniana, C. coniocraea, C. cyanipes, C. deformis, C. fimbriata, C. gracilis* var. *chordalis, C. incrassata, C. macilenta, C. mitis, C. pityrea, C. pleurota, C. Robbinsii, C. submitis, C. subtenuis, C. sylvatica, C. uncialis.* Species which were active against *S. aureus* but not inhibitory for *B. subtilis* are *C. cryptochlorophaea* and *C. gonecha.*

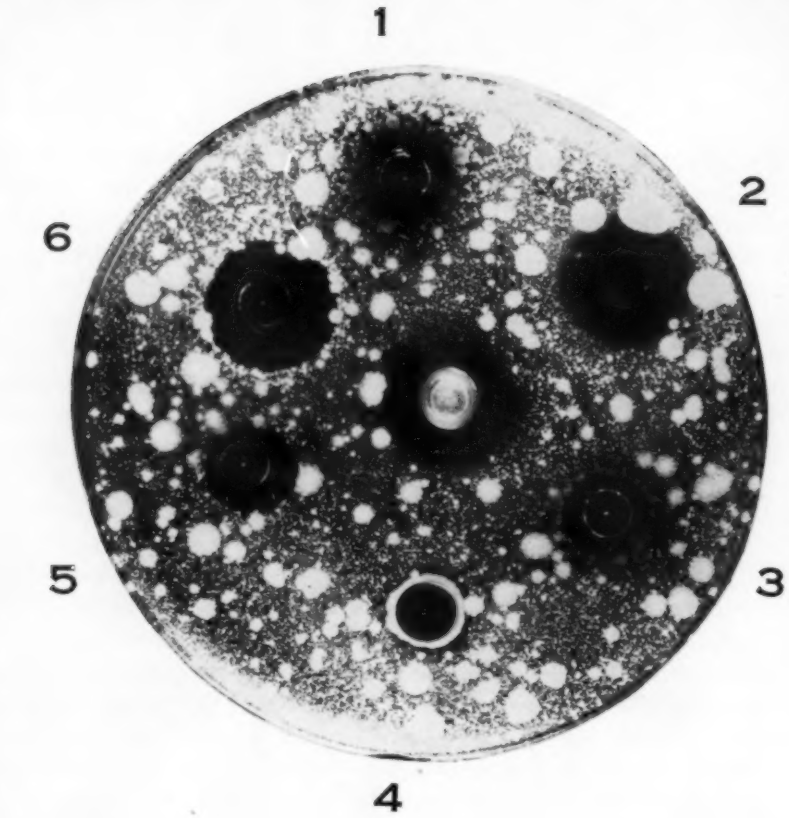
The occurrence of various characteristic acids in these species of *Cladonia* has been considered from the standpoint of a possible explanation for the observed antibacterial activities. The published work of several investigators provides information in this field (4, 5, 6, 7). In the group of 35 active species of *Cladonia,* characteristic compounds are found with varying frequency in the different kinds of lichens. Usnic acid occurs in 17 of the species, fumarprotocetraric acid in 14, squamatic acid in 7, substance A in 6, substance F in 6, barbatic acid in 6, didymic acid in 4, substance B in 3, substance C in 2, substance E in 2, baeomycic acid in 2, psoromic acid in 2, Zeorine in 2, bellidiflorine in 2, grayanic acid in 2, and substance D, substance G, strepsiline, cryptochlorophaeic acid, perlatolic acid, merochlorophaeic acid, homosekikaic acid, thamnolic acid, and atranorine each in one but not the same species. Although we have only meagre evidence for the theory that antibiosis in lichens is related to the constituent diagnostic compounds, still the structural similarity between known inhibitory substances and certain lichen acids is highly suggestive. The possible use of this type of information will be illustrated with one example.

Three typical lichen compounds, atranorine, fumarprotocetraric acid, and usnic acid, were obtained from vials accompanying herbarium specimens of Sandstede's *Cladoniae exsiccatae* in Yale University. Cylinder plate tests indicated that usnic acid is active against *B. subtilis* but not against *S. aureus* or *E. coli*. Usnic acid, crystallized from a species of *Usnea* and supplied to us by Prof. Werner Bergmann, likewise showed activity in tests with *B. subtilis*. Usnic acid is practically insoluble in water, and for this reason a saturated solution with a few minute crystals in excess was employed in making the tests (see figure 2). Atranorine and fumarprotocetraric acid were inactive against the three bacteria used in these tests.

TABLE 1. *Comparative antibiotic activity of extracts from 11 species of lichens tested against 13 kinds of bacteria. Diameter of inhibition zone is given in mm. Diameter of cup is 8 mm.*

Species of bacteria	Species of lichens										
	Usnea florida	*Stereocaulon paschale*	*Parmelia physodes*	*Parmelia caperata*	*Cladonia uncialis*	*Cladonia sylvatica*	*Cladonia pleurota*	*Cladonia mitis*	*Cladonia cristatella*	*Cetraria nivalis*	*Cetraria cucullata*
Staphylococcus aureus	0	17	10	13	0	0	0	0	0	0	0
Staphylococcus albus (hemolytic)	0	0	11	0	12	10	13	12	9	0	12
Diplococcus pneumoniae	0	0	0	11	12	12	15	14	9	0	12
Streptococcus hemolyticus	0	0	0	12	10	0	10	10	0	0	10
Streptococcus viridans	0	0	0	15	9	0	12	11	0	0	11
Bacillus subtilis	17	20	10	24	26	26	29	24	21	15	22
Bacillus mycoides	16	12	0	17	19	19	20	20	16	17	21
Sarcina lutea	30	13	12	25	25	34	32	28	38	40	36
Serratia marcescens	0	0	0	0	0	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0	0	0	0	0	0
Alcaligenes fecalis	0	0	0	0	0	0	0	0	0	0	0
Aerobacter aerogenes	0	0	0	0	0	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0

Diagnostic compounds known to occur in the antibiotic species of *Cladonia* have been listed but other unidentified substances might be responsible for the observed antibacterial properties. Many species which do not contain usnic acid are capable of inhibiting *B. subtilis*, and this acid does not explain the inhibition of *S. aureus* by many lichens. Although completely unknown substances remain to be discovered in these primitive plants, still it seems probable that the preparation of a series of characteristic compounds from antibiotic lichens might provide a means of identifying the substances which are the basis for chemical antagonisms observed in our survey. Following the clear demonstration of antibacterial activity exhibited by lichen sub-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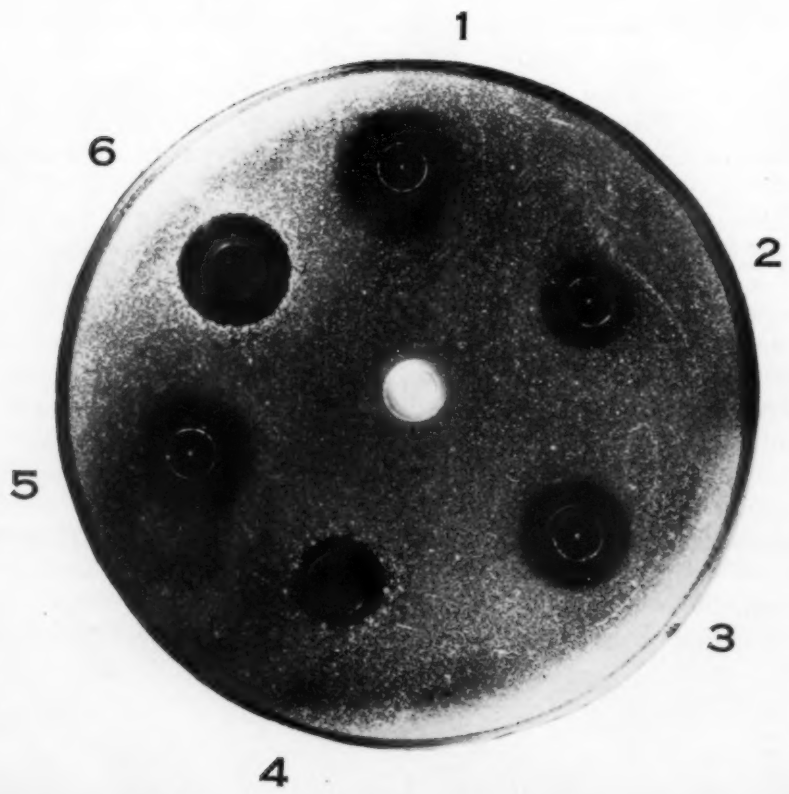
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stances of known chemical structure, there would remain for the organic chemist the attractive possibility of preparing derivatives with selective activity against different kinds of protoplasm. Investigations in this field have been initiated in the Yale laboratories.

The species of lichens for which activity against *B. subtilis* is illustrated in figure 1 were tested along with other lichens against additional species of bacteria. Typical data are presented in table 1. In this group of lichens no activity was found against the five kinds of Gram-negative bacteria listed. All of these lichens were active against *B. subtilis* and *Sarcina lutea*. In a few tests made with the anaerobe *Clostridium Welchii*, extracts of *Cladonia mitis* and *C. pleurota* were found inhibitory. *Lactobacillus casei* was strongly inhibited by several of the species of *Cladonia* which were active against *B. subtilis*. Comparatively small zones of inhibition were observed for the Gram-positive pathogenic bacteria.

When tested against Gram-negative bacteria, most lichens appear to have little or no antibiotic activity. In a few tests made with the Gram-negative *Alcaligenes fecalis*, the following lichens exhibited some activity: *Cladonia delicata*, *C. glauca*, *Parmelia conspersa*, *P. rudecta*, *Thamnolia vermicularis*, and *Xanthoria parietina*. Active against *E. coli* were *C. borbonica* and *P. rudecta*, and against *Proteus vulgaris* the lichens *Parmelia physodes* and *Umbilicaria papulosa* showed slight inhibition.

In addition to the lichens already listed in table 1 or mentioned in the discussion, other species which were found to be antibiotic for either *S. aureus* or *B. subtilis* or for both of these bacteria are the following: *Alectoria chalybeiformis* (variable against *B. subtilis*), *Baeomyces aeruginosa*, *Cetraria juniperina*, *C. Oakesiana*, *Dactylina arctica*, *Dermatocarpon miniatum*, *Evernia vulpina*, *Lepra* sp., *Nephromopsis ciliaris*, *Parmelia conspersa*, *P. rudecta*, *Physcia* sp., *Ramalina* sp., *Rhizocarpon* sp., *Sphaerophorus globosus*, *Thamnolia vermicularis*, *Umbilicaria papulosa*, and various species of *Usnea*. The sample of *Evernia vulpina*, contributed by Dr. Werner Bergmann, came from material which had been stored for a number of years in the Department of Chemistry at Yale University.

Species of lichens which were found inactive when tested against *S. aureus* and *B. subtilis* are the following: *Baeomyces placophyllus*, *B. roseus*, *Cetraria islandica*, *Cladonia apodocarpa*, *C. chlorophaea*, *C. clavulifera*, *C. conista*, *C. delicata*, *C. furcata*, *C. gracilis* var. *dilatata*, *C. gracilis* var. *elongata*, *C. mateocyatha*, *C. ochrochlora*, *C. rangiferina*, *C. santensis*, *C. scabriuscula*, *C. squamosa* var. *denticollis*, *C. subcariosa*, *C. turgida*, *C. verticillata*, *Lobaria pulmonaria*, *Parmelia Cladonia*, *Peltigera variolosa*, *Umbilicaria*

Explanation of figure 2

Antibacterial action of usnic acid and extracts from six species of lichens. Above, *S. aureus*; below, *B. subtilis*. The cup in the center of each plate contained usnic acid. Peripheral cups contained extracts from the following lichens: 1, *Cladonia caespiticia*; 2, *C. cristatella*; 3, *Parmelia physodes*; 4, *C. strepsilis*; 5, *C. capitata*; 6, *C. Grayi*.

Dillenii, and *Xanthoria parietina*. Several unidentified specimens belonging in the genera *Cetraria* and *Parmelia* were also inactive. It is possible that other specimens of these lichens might be found active against these or other test organisms. As an example, many samples of *Cladonia rangiferina* were observed to be inactive against *B. subtilis* and *S. aureus*, but when tested against *Sarcina lutea* some antibiotic activity was shown (see figure 1).

Our observations indicate the presence of antibacterial substances in numerous species of *Cladonia* and in representatives of other genera of lichens. Among these primitive plants, chemical antagonism appears to be a defense mechanism against Gram-positive airborne bacteria. Whether the active agents are to be regarded as bactericidal or merely bacteristatic is not proved. Transfers of agar suspensions from the inhibition zones of *S. aureus* test organisms which had been treated with extracts from *Cladonia cristatella*, *C. cryptochlorophaea*, *C. Grayi*, *C. coniocraea*, and *Parmelia physodes* grew well in fresh nutrient agar. Transfers of *B. subtilis* which had been inhibited by similar extracts failed to grow in nutrient agar or broth. It may be that the inhibitory substances produced by lichens prevent temporarily the growth of some microorganisms and actually kill others. The phenomenon of antibiosis, which seems to be widespread in nature and of great significance as a factor in ecology, is well exemplified in the lichens.

SUMMARY

Of the approximately one hundred kinds of lichens tested for antibiotic activity against bacteria, fifty-two were found to inhibit either *Bacillus subtilis* or *Staphylococcus aureus* or both of these species. Although Gram-positive bacteria including several pathogenic types are inhibited, Gram-negative bacteria with a few exceptions are generally not susceptible to the antibiotic substances of lichens. Usnic acid, obtained from *Cladonia mitis* and *Usnea florida*, inhibited *B. subtilis*. The significance of other characteristic compounds known to be present in lichens is discussed in relation to their possible antibacterial activity.

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A SURVEY OF SOME WOOD-DESTROYING AND OTHER FUNGI FOR ANTIBACTERIAL ACTIVITY[1]

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Fleming's discovery (1) of the production of penicillin by *Penicillium notatum*, and its development as an important therapeutic agent, have stimulated new interest in the antibiotic activity of the fungi. Reviews of earlier work have been published by Waksman (4, 5), Porter and Carter (2), and Weindling (6). Wilkins and Harris (7) have reported on the production of bacteriostatic substances by about 100 fungi belonging mainly to the genera *Penicillium* and *Aspergillus*. This paper presents a survey of the antibacterial activity of over 300 wood-destroying fungi, 22 dermatophytes, and about 50 miscellaneous fungi against *Staphylococcus aureus* and *Escherichia coli*. The antibiotic effects for any fungus are influenced by a number of factors, including the strain of the fungus, the medium used for its cultivation, the length of the growth period, the organism used in assay, and the method of assay. The results in this paper should not be considered as absolute, but preliminary and suggestive for later and more detailed investigation.

Preliminary Experiments, Miscellaneous Fungi. In some preliminary experiments[2] a modification of Fleming's streak method of assay (1) was used for testing the antibacterial activity of a group of miscellaneous fungi. The fungus to be tested was inoculated at the side of a Petri dish, 15×100 mm., containing 15 ml. of an agar medium and grown at 25° C until it had reached a diameter of 2 or 3 cm. or, if it grew slowly, until it was a week or more old. The bacteria (a broth culture from 1 to 10 days old) were then streaked up to the edge of the fungus colony. *Staphylococcus aureus* and *Escherichia coli* were used as test organisms because they represented gram-positive and gram-negative organisms respectively. The streaked plates were incubated overnight at $37 \pm 2°$ C and inspected for the absence of bacterial growth adjacent to the fungus colony.

The medium on which the fungi were grown contained per liter 10 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, 1 g. neopeptone, 1000 mμ moles thiamine, and 20.0 g. of Difco agar.[3] It was adjusted to neutrality with NaOH.

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[2] These experiments were performed in June, 1943.

[3] Mineral supplements in p.p.m. were added as follows: 0.005 B, 0.02 Cu, 0.10 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn.

Results with Streak Method for Miscellaneous Fungi. No inhibition or doubtful results were obtained with the following fungi:

Absidia orchidis (+ and −)
Absidia ramosa (+ and −)
Ashbya gossypii
Cephalosporium acremonium
Ceratostomella ips (2 isolations)
Ceratostomella fimbriata
Ceratostomella from London plane tree
Ceratostomella microspora
Ceratostomella minutum
Ceratostomella montium
Ceratostomella multiannulata
Ceratostomella obscura
Ceratostomella paradoxa (3 isolations)
Ceratostomella penicillata
Certostomella piceaperda
Ceratostomella pilifera
Ceratostomella pini (2 isolations)
Ceratostomella pseudotsugae
Ceratostomella radicicola
Ceratostomella stenoceras
Chalaropsis thielavioides (3 isolations)
Claviceps purpurea
Endoconidiophora adiposa
Endoconidiophora coerulescens (2 isolations)
Fusarium avenaceum (5 strains)
Gibberella saubinetti
Gibberella zeae (2 isolations)
Grosmannia serpens (2 isolations)
Mucor mucedo
Mucor ramannianus
Neurospora crassa
Neurospora sitophila
Ophiobolus oryzinus
Ophiostoma catonianum
Pholiota adiposa
Phycomyces blakesleeanus (+)
Phytophthora cinnamomi
Polyporus versicolor
Pythiomorpha gonapodyides
Pythium butleri
Pythium helicoides
Rhizopus nigricans (+ and −)
Schizophyllum commune
Sclerotinia from eggplant
Sclerotinia from opuntia
Sporotrichum globulum
Trichophyton gypseum

Aspergillus niger, *Ceratostomella rostrocylindrica*, *C. ulmi*, *Sclerotium delphinii*, *S. rolfsii*, and *Stereum murrayi* produced antibiotic substances which inhibited the growth of *Staph. aureus* or *E. coli* for distances ranging from 3.0 to 15 mm. from the edge of the fungus colony. Three of these fungi (*Aspergillus niger* and the two species of *Sclerotinia*) produced considerable acid as judged by indicator paper, and it appeared probable that their antibiotic activity was to be accounted for by the lowering of the hydrogen-ion concentration in the vicinity of the fungus colony. *Stereum murrayi* and the two species of *Ceratostomella* had little effect on the reaction of the medium. Their antibiotic activity appeared to be caused by other substances than the hydrogen-ion.

Antibiotic Activity of Wood-destroying Fungi and Dermatophytes.[4] Since three wood-inhabiting fungi included in the preliminary experiments evidenced antibiotic activity not accounted for by acid produced by the organisms, it seemed desirable to survey a considerable number of this group.

The extensive collection of wood-destroying fungi in the Division of Forest Pathology of the U. S. Department of Agriculture was made avail-

[4] Most of the fungi, except the dermatophytes, included in this portion of the survey are wood-destroyers. Some, however, for example *Morchella esculenta*, are not.

able to us. These are distinguished later in this paper by the stock numbers given with each fungus. Some of these numbers represent cultures from other workers in the United States and from the Department of Agriculture, Ottawa, Canada, which were received through exchange. Those marked (CBS) are from Centraal Bureau voor Schimmelcultures. In addition, Dr. Dow V. Baxter furnished us with some from his collection; these are marked by the letter (B). We included also a *Verticillium* and a *Volutella* from Dr. B. O. Dodge, and a group of 22 dermatophytes obtained from the American Type Culture collection (ATC), through the courtesy of the National Institute of Health (NIH), from Dr. R. Benham (RB), or isolated by ourselves.

Survey of Wood-destroying Fungi by the Streak Method. The procedure for surveying the antibiotic activity of the wood-destroying fungi and dermatophytes was similar to that used in the preliminary experiments described above. The fungi were grown at 25° C in Petri dishes, 15 × 100 mm., containing 15 ml. of 2 per cent malt agar or a thiamine-peptone agar.[5] At times ranging from 3 to 22 days after inoculation, depending on the rapidity with which the fungus grew, the plates were streaked with *Staph. aureus* and *E. coli* (a 24-hour broth culture) from the edge of the colony to the periphery of the dish, a procedure which was considered preferable to that used in the preliminary experiments. Outlining the fungus colony with wax pencil on the bottom of the dish fixed its limits at the time of streaking. The radius of the fungus colony was measured, and as a rule each fungus was tested at two different ages. *Staph. aureus* grew poorly on the 2 per cent malt. The agar in each plate of this medium was, therefore, coated with 10 ml. of a modified thiamine-peptone agar[6] before the streaking with bacteria. The plates were incubated for 24 hours at 37 ± 2° C and examined for evidence of inhibition of the growth of the bacteria. When inhibition was observed the distance from the edge of the fungus colony to the bacterial growth was measured.

Results with Wood-destroying Fungi on Streak Plates. One hundred and twenty-three of the fungi showed no inhibition of *Staph. aureus* or *E. coli* either on the thiamine-peptone medium or on the malt agar. These were as follows:

[5] The malt agar was prepared by adding 2 per cent desiccated malt extract to 1.5 per cent Difco agar. The pH of this medium was 5.0–5.4. The thiamine-peptone agar contained per liter, 10 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, 1.0 g. neopeptone, 15 g. Difco agar, and 600 mμ moles of thiamine. The mineral supplements given earlier were also added. The pH of this medium was 5.5.

[6] This consisted of the thiamine-peptone agar plus 1000 mμ moles of nicotinamide per liter, and the KH_2PO_4 replaced with a mixture of 1.069 g. $Na_2HPO_4 \cdot 2H_2O$ and 0.545 g. KH_2PO_4. The pH of this medium was close to 7.0.

Collybia velutipes (55001)
Collybia radicata (72084–R)
Coprinus radians (S–249)
Corticium cremoricolor (57082–R)
Corticium salmonicolor
Cytidia salicina (15184–Sp)
Daedalea ambigua (57066–S)
Daedalea confragosa (67939–S)
Daedalea unicolor (72082 and 72153)
Exidia glandulosa (72147–S)
Favolus canadensis (52003)
Fomes calkinsii (14667–S)
Fomes connatus (71522–R)
Fomes densus (52046–S)
Fomes everhartii (57210–S and B)
Fomes fomentarius (59010–S)
Fomes fraxineus (50357–R)
Fomes igniarius (86391)
Fomes igniarius var. *laevigatus* (94020)
Fomes rimosus (59124–S and CBS)
Fomes robustus (71987–S)
Fomes robustus var. *tsugina* (16836–S)
Fomes torulosus (179)
Fomes sp. (59163–R)
Hydnum chrysocomum (1605)
Hydnum ochraceum (71953)
Hydnum pulcherrimum (50343–S)
Lentinus tigrinus (50390–R)
Lentinus villosa (57001)
Lenzites betulina (13813–R)
Lenzites sp. (1682)
Lenzites trabea (539)
Merulius niveus ? (71734–R)
Merulius tremellosus (94184)
Morchella esculenta
Panus laevis (71178–R)
Panus rudis (1927)
Panus strigosus (55699–S)
Peniophora allescheri (56483–S)
Peniophora gigantea (56475–S)
Peniophora pubera (71412–R)
Pholiota adiposa (59116–S)
Pholiota aurivella (CBS)
Pholiota limonella (71894–S)
Pholiota spectabilis (72080)
Pleurotus corticatus (75056–S)
Pleurotus ostreatus (56478–S)
Pleurotus sapidus (71958)
Pleurotus ulmarius (71386–S)
Polyporus abietinus (71429–R)
Polyporus adustus (56476–S)
Polyporus anceps (71825–S and 58526–R)
Polyporus arcularius (30680)
Polyporus berkeleyi (53379–S)
Polyporus brumalis (56439–S)
Polyporus cinnabarinus (57071–S)
Polyporus cuticularis (71342–S)
Polyporus dichrous (57474–S)
Polyporus distortus (57059–S)
Polyporus dryadeus (71092–S)
Polyporus dryophilus (71539–R)
Polyporus ectypus ? (81034–R)
Polyporus fibrillosus ? (94071–R)
Polyporus fissilis (50384–S)
Polyporus frondosus (50222 and 72076–S)
Polyporus fumosus (71335–S)
Polyporus gilvus (56480–S)
Polyporus glomeratus (71126)
Polyporus graveolens (71021)
Polyporus hirsutus (56479–S)
Polyporus hispidus (59106–S and 43 Findlay)
Polyporus hydnoides (80938–R)
Polyporus lucidus (50390–S)
Polyporus mutabilis (57069–S)
Polyporus osseus (551)
Polyporus pargamenus (57403–S)
Polyporus picipes (59060–R)
Polyporus pinsitus (71973–R)
Polyporus pubescens (71448–R)
Polyporus robiniophilus (67904)
Polyporus resinosus (B)
Polyporus rutilans (2287)
Polyporus spumeus (71929–S)
Polyporus spumeus var. *malicola* (18565–S)
Polyporus squamosus (CBS)
Polyporus subectypus (57006)
Polyporus supinus (57014–R)
Polyporus tenuis (57012)
Polyporus texanus (94178–R)
Polyporus velutinus (15067)
Polyporus versicolor (71753–R)
Polyporus zonalis (55571)
Polyporus zonatus (CBS)
Poria ambigua (86357)
Poria ferruginosa (B)
Poria inermis (B)
Poria nigrescens (52041–S)
Poria obliqua (58561–S and 59134–S)
Poria prunicola (B)
Poria spiculosa (94102)
Poria weirii (B)
Ptychogaster cubensis (300)
Schizophyllum commune (56473–S)
Stereum albobadium (71523–Sp)
Stereum hirsutum (71556–Sp)
Stereum ochraceo- flavum (123)
Stereum pini (14780–R)
Stereum purpureum (59171–R)
Stereum sanguinolentum (1292–S)
Stereum spadiceum (71427–Sp)
Trametes cubensis (55613–S)
Trametes hispida (68583–S)
Trametes rigida (57004–R)
Trametes suaveolens (71404–S)
Verticillium buxi (D)
Volutella buxi (D)

With the following fungi zones of inhibition of less than 5 mm. were observed on the thiamine-peptone agar for *Staph. aureus:*

Armillaria mellea (70973–R)
Clitocybe tabescens (512)
Corticium coeruleum (14909)
Corticium confluens (71544–R)
Corticium galactinum (72033)
Corticium roseum (56489–S)
Fomes clelandii (CBS)
Fomes lobatus (71656–R)
Hymenochaete curtisii (71519–Sp)
Lenzites trabea (B)
Lycoperdon gemmatum (52001)
Lycoperdon pyriforme (64801)
Pholiota lutea (71389–S)
Polyporus croceus (47479–S)
Polyporus oregonensis (1273)
Polyporus schweinitzii (94095–R)
Polyporus sulphureus (71371–S)
Polyporus varius (2290)
Poria ferruginosa (B)
Poria inflata (94089–S)
Poria mutans (72029–S)
Poria prunicola (94048–S)
Poria punctata (50266–R)
Stereum frustulosum (24059)
Stereum rameale (86400)
Stereum roseo-carnum (57416–S)
Stereum rufum ?
Stereum sanguinolentum (71776–R)
Stereum sericeum (58500–R)

With the majority of this group the antibiotic activity was limited to *Staph. aureus* on the thiamine-peptone agar; there was none on malt agar, and none for *E. coli* on either kind of agar. *Polyporus varius* (2290) inhibited *E. coli* and *Staph. aureus* on the thiamine-peptone agar. *Corticium roseum* (56489–S), *Lycoperdon pyriforme* (64801), and *Stereum rameale* (86400) produced a slight zone of inhibition of *Staph. aureus* on both media. For *Daedalea unicolor* (72153) and *Lycoperdon gemmatum* (52001) a slight inhibition of *Staph. aureus* was observed on malt agar, but none on the thiamine-peptone medium.

The following 57 fungi produced zones of inhibition for *Staph. aureus* which ranged between 5 mm. and 10 mm. as a maximum:

Clitocybe illudens (72027–S)
Clitocybe investiens (56445–R)
Echinodontium tinctorium (80517–S)
Fomes conchatus (1403)
Fomes extensus (CBS)
Fomes fulvus (16870–S and CBS)
Fomes geotropus (55521–S)
Fomes igniarius var. *populinus* (59024–S and A8)
Fomes marmoratus (50332–S)
Fomes pomaceus (2283)
Fomes rimosus (53383–S)
Fomes scutellatus (1952–S)
Hydnum coralloides (59062–S and CBS)
Hydnum erinaceus (55195–S)
Hymenochaete agglutinans (71707–R)
Hymenochaete rubiginosa (1650)
Lentinus lepideus (F–1334)
Lenzites saepiaria (1–31–36)
Lenzites trabea (B)
Merulius confluens (15006–R)
Peniophora cinerea (57406–S)
Peniophora coccineofulva (57418–S)
Peniophora incarnata (56449–S)
Peniophora nuda (56494–S)
Pholiota spectabilis (71463)
Polyporus borealis (91430)
Polyporus circinatus (72026–a and 72026–S)
Polyporus compactus (71339–S)
Polyporus cristatus ? (71774–S)
Polyporus curtisii (57051–S)
Polyporus dryophilus var. *vulpinus* (71123)
Polyporus ludovicianus (55537–S)
Polyporus pargamenus (71954–S)
Polyporus schweinitzii (86353)
Polyporus spraguei (72025–S)
Polyporus tuberaster (2345)
Polyporus tuckahoe (*tuberaster* ?) (72176)
Polyporus volvatus (14606)
Poria andersonii (72121–S)
Poria carbonica (8444)
Poria ferruginosa (71181)

Poria inflata (72160–R)
Poria obliqua (58567–S)
Poria undata (P–151)
Poria unita ? (71598)
Poria sp. (142)
Stereum frustulosum (57048)
Stereum gausapatum (58555–S)
Stereum rameale (57046)
Stereum rugosiusculum (71516–Sp)
Stereum subpileatum (58597–R)
Stereum sulcatum (71690–R)
Stereum umbrinum (52060)

With the majority of this group antibiotic activity was limited to *Staph. aureus* and usually on the thiamine-peptone agar and not on malt. Seventeen were observed to inhibit *E. coli* on the thiamine-peptone medium though in almost all instances the zone of inhibition was less than for *Staph. aureus*. These were *Clitocybe illudens* (72027–S), *Fomes fulvus* (16870–S and CBS), *F. pomaceus* (2283), *Hydnum coralloides* (59062–S and CBS), *H. erinaceus* (55195–S), *Lentinus lepideus* (F–1334), *Lenzites saepiaria* (1–31–36), *L. trabea* (B), *Peniophora cinerea* (57406–S), *P. incarnata* (56449–S), *Polyporus schweinitzii* (pine) (86353), *P. sprague*i (72025–S), *Poria carbonica* (8444), *P.* sp. (142), and *Stereum subpileatum* (58597–R).

One hundred and nineteen of the 332 fungi were observed to produce zones of inhibition for *Staph. aureus* of more than 10 mm. on one or both of the media used in the survey. The results are tabulated in table 1. The age in days of the fungus colony when the antibiotic activity was tested is given in column 2; the radius of the fungus colony on malt-agar in column 3; the inhibition zone measured from the edge of the fungus colony for *Staph. aureus* and *E. coli* in columns 4 and 5; the radius of the fungus colony on thiamine-peptone agar and the inhibition zones on that medium in columns 6, 7, and 8. An "A" shows those instances where we believe the acid produced by the fungus was considerable and the inhibition was caused by hydrogen-ion concentration. In all the experiments with the streak method of investigating the antibacterial activity of these fungi the acidity next the fungus colony was tested by applying a piece of indicator paper to the agar. A similar test at the edge of the dish enabled us to approximate the change produced by the fungus. In some instances the indicator paper was supplemented by applying drops of indicator solution to the agar. Most of the fungi which were found by these tests to form considerable acid also developed a zone of precipitation in the medium adjacent to the colony.

As a rule the inhibition of *Staph. aureus* exceeded that of *E. coli*. Again there are exceptions on both media and almost all of these were fungi which produced a great deal of acid. Example, *Poria luteofibriata* (B).

Thirty-six of the fungi were observed to inhibit *Staph. aureus* on the thiamine-peptone agar but to have no or a slight (1 mm.) effect on *E. coli*. Example, *Corticium abeuns*. On malt agar this was observed for 40 of the 119 fungi.

With 40 of the fungi an inhibition of *Staph. aureus* was observed on the thiamine-peptone agar, but none or a slight inhibition (1 mm.) on malt agar. Example, *Corticium lividum* (5159).

While some strains of the same species showed similar responses, among strains of other species considerable differences were observed. See the 6-day-old cultures of *Poria luteofibriata*.

Usually the antibiotic activity increased with the age of the fungus colony, but in some instances it decreased. See *Fomes applanatus* (59054–S).

The character of the inhibition varied. Five types were distinguished as follows: The bacterial streak ended in a sharp distinct line; the streak gradually faded out; the streak ended with a sharp break but isolated colonies appeared between the break and the fungus colony; the streak ended with a sharp break but faint growth gradually fading out continued toward the fungus colony; the streak ended with a sharp break and faint but uniform growth continued up to the fungus colony.

Results with Dermatophytes on Streak Plates. Of the dermatophytes tested 18 showed no inhibition or little inhibition (6 mm. or less) when tested after from 3 to 19 days, depending upon the rapidity of growth. These were *Achorion schoenleini* 4822 (ATC), *A. schoenleini* 2044 (NIH), *Epidermophyton inguinale* (RB), *Microsporum felineum* (RB), *M. felineum* 8137 (ATC), *M. gypseum* (RB), *Trichophyton crateriforme* (RB), *T. gypseum*, *T. gypseum* 2035 (NIH), *T. gypseum* (RB), *T. interdigitale* 4807 (ATC), *T. interdigitale* 4808 (ATC), *T. mentagrophytes* V10 (NIH), *T. purpureum* AMS–3 (NIH), *T. rosaceum* (RB), and *T. tonsurans* V13 (NIH), *T. violaceum* 2034 (NIH), 8532 (ATC) and (RB).

Four of the dermatophytes gave zones of inhibition of 10 mm. or more (table 1) for *Staph. aureus*. *Epidermophyton inguinale* (RB) was quite active on malt agar, and *Microsporum canis* (650–ATC) inhibited *Staph. aureus* on malt but not on thiamine-peptone agar. None was active for *E. coli*.

The Disc Method. The determination of antibiotic activity by the streak method described above is useful chiefly in eliminating a considerable number of the fungi included in a survey. It is possible that those organisms tested by the streak method which evidenced no antibiotic activity, or little activity, when grown on the thiamine-peptone medium, or on 2 per cent malt, would produce antibiotic material effective against the test bacteria if grown on other media, or under other conditions. It is possible also that the fungi inactive against *Staph. aureus* and *E. coli* form substances antibiotic for other organisms. Furthermore, the streak method as used here would not detect organisms which formed antibiotic substances and retained them within the hyphae. However, from a practical standpoint in this survey it seemed most profitable to select for more detailed examination some of

TABLE 1. *Antibiotic activity of fungi tested by streak method*
Zone of inhibition over 10 mm. (p) indicates partial inhibition. See text for details.

Fungus	Age of colony days	Malt agar			Thiamine-peptone agar			Acidity
		Radius colony mm.	Inhibition mm.		Radius colony mm.	Inhibition mm.		
			Staph. aureus	*E. coli*		*Staph. aureus*	*E. coli*	
Claudopus nidulans (71364–S)	9	17	9	0	10	13	0	....
	15	19–28	3	0	22–25	25	4	....
Coniophora cerebella (94042–R)	6	11	5	5	8	25	26	....
	9	17	0	0	9–18	40	33	A
C. suffocata (56488–S)	5	20	6–7	1	18	13	9	....
	6	....	8	1	....	12	12	....
Corticium abeuns ? (14572)–R	6	8	29	0	10	29	0	....
	6	7.5	34	0	10	25	0	....
C. conigenum (66211–Sp)	4	13	5	0	12	7	1	....
	6	28	0	0	25	11	0	....
C. hydnans (57407–S)	6	10	16	2	7	31	12	....
	10	18	21	5–6	11	43	21	....
C. lividum (5159)	7	26	0	0	7	15	10	....
	7	34	0	0	5	20	10–13	....
C. lividum (15084–R)	6	30	0	0	....	....	....	....
	15	....	....	....	....	32	26	....
Daedalea heteromorpha (59051–R)	6	5.5	21	0	5.5	27	10–15	....
	9	15	7–11	20	13	45	27	A
D. juniperina (71585–R)	6	10	10	6(p)	10	20	15(p)	....
	9	21	11	10(p)	19	31	25(p)	A
D. quercina (57076–S)	5	13	6	0	12	15	8	....
	6	....	10	8 (p)	....	20	19–20	A
Fistulina hepatica (1818)	7	....	0	0	....	3	0	....
	14	18	0	0	12	26	0	....
Flammula alnicola (2071)	6	15	4	0	12	19	11	....
	11	24	9	3	15–18	36	21	....
Fomes annosus (58548–S)	6	32	5	0	29	20–27	0	....
	6	37	3	0	25	10–15	0	....
F. applanatus (59054–S)	7	6	0	0	6	10–12	10–15	....
	10	10	0	0	9	3(p)	0	....
F. ferruginosa ? (H–43)	10	2–4	0	0	2–4	11	0	....
	19	10	0	0	9	0	0	....
F. fraxinophilus (59120–S)	8	10	0	0	16	13	0	....
	13	13	0	0	15	12	0	....

TABLE 1 (*continued*)

Fungus	Age of	Malt agar			Thiamine-peptone agar			
		Radius	Inhibition mm.		Radius	Inhibition mm.		

TABLE 1 (*continued*)

Fungus	Age of colony days	Malt agar			Thiamine-peptone agar			Acidity
		Radius colony mm.	Inhibition mm.		Radius colony mm.	Inhibition mm.		
			Staph. aureus	*E. coli*		*Staph. aureus*	*E. coli*	
F. fraxinophilus (B)	6	15	0	0	12	9	0	....
	9	28	0	0	15	20	8	....
F. juniperinus (72028–S)	13	8	28	18	6	41	35	....
	19	8	32	25	7	5	1	....
F. juniperinus (CBS)	13	9	14	6	7	27	22	....
	13	9	13	6	7	28	22	....
F. meliae (50336–R)	4	13	8	3	12	20	15	....
	8	25–28	13	5	17–19	35	25	A
F. officinalis (80518–S)	10	8–9	10–12	3(p)	5	28	12	....
	13	10	21	4	8	50	27	A
F. pini (52061–S)	6	10	3	0	8	18	10	....
	8	13	6	5	10–11	26	15	....
F. pini var. *abietis* (71112)	8	12	0	0	9	7	0	....
	10	15–17	5–6	1–2	13–14	21	12	A
F. pinicola (59017–S)	6	15	5	0	15	28	25	....
	8	23	13	5	22	37	32	A
F. ribis (94142)	10	7	10(p)	0	10–11	15	0	....
	13	10	8	0	12	46	0	....
F. ribis (CBS)	5	....	0	0	....	6	3	....
	6	16	2	0	13	12	3	....
F. roseus (10)	6	8	5	0	13	13	10(p)	....
	8	15	0	0	22	12	2(p)	....
F. subroseus (20)	4	10	0	0	9	17	10	....
	6	20	0	0	19	23	18	A
F. tenuis (58528–R)	10	8–9	0	0	5–9	10	0	....
	13	11	0	0	10	11	0	....
F. ulmarius (CBS)	7	....	0	0	....	40	36	....
	10	8	0	0	4	20–25	18(p)	A
F. ungulatus (CBS)	6	24	10(p)	4(p)	10	28	25	....
	8	27	10	9	15	40	30	A
Ganoderma sp. (86194)	4	15	0	0	9	9	0	....
	6	29	0	0	20	13	0	....
Hirneola sp. (Iowa 42)	6	....	0	0	....	0	0	....
	14	....	....	....	....	18	2	....
Hydnum abietis ? (3548)	16	14	22	0	6	44	1	....
	....	....	....	....	....	....	....	....

TABLE 1 (*continued*)

Fungus	Age of colony days	Malt agar			Thiamine-peptone agar			Acidity
		Radius colony mm.	Inhibition mm.		Radius colony mm.	Inhibition mm.		
			Staph. aureus	*E. coli*		*Staph. aureus*	*E. coli*	
H. caput-ursi (71748)	7	14	0	0	9–11	14	8	....
	16	17–28	8	5	13–19	20	14	....
H. scheidermayeri (71787–R)	7	....	6	0	....	28	3(p)	....
	14	18–22	3(p)	0	8	40	15(p)	....
H. septentrionale (52037–S)	7	18	4	0	12	13	0	....
	16	35	6	0	25–28	20	6	....
H. septentrionale (72067)	7	18	0	0	13	4	0	....
	7	18	0	0	13	15	0	....
Hymenochaete corrugata (71759–R)	5	13	7	2–3(p)	14	13	4–5(p)	....
	6	....	....	....	....	15	7(p)	....
H. rubiginosa (58557–R)	5	4	0	0	....	....	....	....
	14	17	0	0	6–7	30	0	....
H. rubiginosa (58512–R)	6	....	3(p)	0	....	3	0	....
	14	15	5	0	16–17	20	0	....
H. tabacina (71248–R)	5	16	0	0	13	10–12	4(p)	....
	6	....	0	0	....	10	5(p)	....
Hypholoma sublateritium (56468–S)	6	10	5	0	9	9	0	....
	11	22	3	0	20	11	0	....
Irpex cinnamomeus (67937)	4	11	0	0	10	7	2	....
	6	17	0	0	15	16	5	....
I. mollis (71542–R)	6	....	15	0	....	25	1	....
	9	35–40	0	0	45	0	0	....
Ithyphallus sp. (S–1)	9	13	0	0	10	9(p)	4(p)	....
	15	21–22	0	0	17–20	27	10	....
Lenzites saepiaria (13812)	6	21	0	0	20	10	0	....
	9	26	3	0	30	13	5	....
Lepiota naucina (312)	9	17	13	8	10	18	13	....
	15	23–27	9	4	16–22	22	9	....
Omphalia campanella (94075–R)	15	12	3	0	7	14	0	....
	22	20	1–2	0	14	12	0	....
Panus stipticus (71518–Sp)	6	10	10	0	10	6	0	....
	11	23	4	0	20	12	0	....
P. stipticus (luminous) (CBS)	9	7	2	0	8	9	0	....
	15	16	0	0	18	11	0	....
Paxillus involutus (94261–S)	10	3–3.5	6	0	11–14	9	0	....
	13	3	0	0	10	16	0	....

TABLE 1 (*continued*)

TABLE 1 (*continued*)

Fungus	Age of colony days	Malt agar			Thiamine-peptone agar			Acidity
		Radius colony mm.	Inhibition mm.		Radius colony mm.	Inhibition mm.		
			Staph. aureus	*E. coli*		*Staph. aureus*	*E. coli*	
Phlebia strigosozonata (56454–S)	4	13	0	0	10	15	0	....
	6	....	0	0	....	25	5	....
Pholiota aeruginosa (1997)	6	13	0	0	11	11	0	....
	9	22	0	0	17	3	0	....
Pleurotus griseus ? (14616–R)	9	7–9	14–17	0	5	26–30	0	....
	15	19	8–16	0	14	43–48	0	....
Polyporus albellus (14554–S)	4	18	0	0	18	12	0	....
	6	27	0	0	26	12	0	....
P. alboluteus (72163)	5	24	0	0	27	15	0	....
	6	30	0	0	32	8	0	....
P. balsameus (71191–R)	6	11	0	0	11	20	10	....
	10	26	0	0	22	29	22	A
P. betulinus (58514–S)	5	34	0	0	23	9	5	....
	6	40	0	0	27	12	0	A
P. biformis (71423–R)	5	27	12	9	27	15	3	....
	6	34	13	2	27	15	3	....
P. delectans (71089)	7	15	6	0	13	25	0	....
	16	44	0	0	17	22	0	....
P. durescens (71096)	4	17	7	5	16	29	26	....
	5	23	10	10	20	30	23	A
P. fragilis (71326–S)	7	10	7	0	6	32	27	....
	10	13	10	5	9	45	40	A
P. fumidiceps (59064–S)	5	28	0	0	25	9	0	....
	6	32	0	0	30	11	0	A
P. galactinus (52034–S)	6	25	0	0	20	13	0	....
	7	43	0	0	37	9	0	....
P. giganteus (50382–R)	7	11	5	0	14	16	0	....
	10	15	18	0	8–9	26	0	....
P. giganteus (57026)	10	8	12	0	5–6	15	7	....
	14	12	9	0	8	25	0	....
P. guttulatus (71799–S)	6	5	0	0	3	0	0	....
	10	14	2	0	9	18	10	A
P. immitus (71384)	7	16	8	3	23	30	23	....
	10	36	9	7	24	32	32	A
P. obtusus (71791–S)	6	13	8	0	7	21	16	....
	10	33	7	0	19	17	0	....

TABLE 1 (*continued*)

Fungus	Age of colony days	Malt agar			Thiamine-peptone agar			Acidity
		Radius colony mm.	Inhibition mm.		Radius colony mm.	Inhibition mm.		
			Staph. aureus	*E. coli*		*Staph. aureus*	*E. coli*	
P. palustris (57010)	5	21	10	0	19	26	20	....
	6	22	7	7	22	32	25	A
P. radiatus (71313–S)	6	15	0	0	11	0	0	....
	10	34	6	0	23	26	0	....
P. radiatus (B)	14	9–10	9	0	10	25	0	....
	21	24	3	0	21–22	23(p)	0	....
P. resinosus (52043–S)	6	8	0	0	4	0	0	....
	10	20–39	0	0	8–20	30	0	....
P. schweinitzii (71356–S)	11	22	0	0	6	46	33	....
	21	31	0	0	19	24	0	A
P. subcartilagineus (234)	18	15	9	8	26	35	33	....
	21	27–38	28	10	....	....	....	A
P. trabeus (55196–R)	4	21	0	0	14	16	0	....
	5	24	0	0	23	14	0	....
P. tulipiferus (71938)	4	36	0	0	41	19	12(p)	....
	4	40	0	0	38	17	11(p)	....
P. vulpinus (2279)	7	16	12	10	10	28	18	....
	7	17	12	8	13	11	0	A
Poria cocos (71693–T)	3	35	0	0	29	17	12	....
	3	34	0	0	28–30	14	11	A
P. colorea (71963–R)	4	10	7	0	11	11	0	....
	6	21	7	0	21	11	0	....
P. corticola ? (71280–R)	4	13	10	0	12	18	0	....
	8	33	10	0	31	25	7	....
P. ferrea (B)	9	11	0	0	13	11	trace	....
	15	21	0	0	18	22	4	....
P. ferruginia-fusca (1411)	4	8	5	0	11	16	0	....
	6	16	6	0	14	25	3	....
P. incrassata (59125–R)	6	11	6	4	11	30	20	....
	8	22	9	1	15	36	24	A
P. incrassata (B)	6	12	0	0	11	20	12	....
	7	24	0	0	18	24	19	A
P. luteofibriata (2996)	6	19	0	0	10	22	20	....
	8	25	6	4	17	25	17	A
P. luteofibriata (B)	6	15	25	20	6	35	35	....
	14	14	24	30	21	55	55	A

TABLE 1 (*continued*)

TABLE 1 (*continued*)

Fungus	Age of colony days	Malt agar			Thiamine-peptone agar			Acidity
		Radius colony mm.	Inhibition mm.		Radius colony mm.	Inhibition mm.		
			Staph. aureus	*E. coli*		*Staph. aureus*	*E. coli*	
P. microspora (575)	6	24	25	23	24	42	37	....
	8	36	9	9	31	50	50	A
P. nigra (71532–R)	6	18	4	0	17	12	5	....
	8	25	1	1	22	10(p)	6	A
P. sequoiae (F–8755)	6	15	6	0	15	16	11	....
	8	18	4	0	20	15	9	....
P. subacida (71155)	6	20	11	0	25	20	3	....
	8	12	9	0	34	20	6	....
P. subacida (B)	6	25	8	0	24	18	0	....
	7	35	5	0	38	18	0	A
P. tenuis (67942)	6	17	13	0	15	35	11	....
	8	23	15	0	20	33	9	....
P. vaillantii (13780)	6	18	12	6	17	30	22	....
	8	23	12	3	17	25	14	A
P. vaporaria (729)	6	13	10	0	14	21	18	....
	8	20	0	0	21	26	20	A
P. weirii (91394–R)	6	17	0	0	16	4	0	....
	8	23	0	0	20	11	0	....
P. xantha (94154–S)	6	30	15	7	23	31	27	....
	6	28	13	4	25	33	27	A
Ptychogaster rubescens (CBS)	6	16	0	0	17	15	3	....
	9	27	0	0	27	16	8	A
P. rubescens (Minnesota)	6	12	0	0	9	11	4	....
	9	23	0	0	21	16	8	....
Radulum orbiculare (57402–S)	10	9	24	9	5–8	46	17	....
	13	11	23	3	8	51	26	....
Sparassus crispa (CBS)	7	....	0	0	....	6	5	....
	14	13	0	0	11	30	12	....
Stereum fasciatum (52006–R)	4	....	....	....	3.5	20	0	....
	9	36	1	0	20	10	0	....
S. fuscum (71509–Sp)	4	9	0	0	6	6	0	....
	9	29	0	0	18	17	0	....
S. gausapatum (58581)	6	4	16	6	5	11	2	....
	11	8	15	5	11	23	12	....
S. gausapatum (77–A)	4	15	3	0	5.5	5	0	....
	12	....	....	....	5	12	5	....

TABLE 1 (*continued*)

Fungus	Age of colony days	Malt agar			Thiamine-peptone agar			Acidity
		Radius colony mm.	Inhibition mm.		Radius colony mm.	Inhibition mm.		
			Staph. aureus	*E. coli*		*Staph. aureus*	*E. coli*	
S. rugosum (86351)	8	6	17	0	6–8	10	0	.....
	13	9	26	0	13	32	4	.....
S. subpileatum (50230–R)	4	.....	.....	.....	3	0	0	.....
	12	25	0	0	6–8	11–15	7	.....
S. sulcatum (71329–R)	4	4	0	0	4	0	0	.....
	12	13–14	14	6	9	14–17	10	.....
Trametes alaskana (B)	11	18	0	0	8	11	5	.....
	15	28	0	0	18	15	5	A
T. americana (18125–R)	6	12	0	0	13	15	6	.....
	9	21	3	0	21	14	6	.....
T. carnea (571)	4	14	0	0	9	6(p)	0	.....
	6	24	5	0	17	17	7	A
T. heteromorpha (71189–S)	6	15	10	0	15	37	30	.....
	9	21	12	13	22	44	40	A
T. malicola (71956)	6	12	4	0	12	25	11	.....
	9	22	0	0	18	33	27	A
T. malicola (B)	6	12	22	22	8	45	37	.....
	9	14	21	23	11	52	50	A
T. rubescens (H–61)	6	14	12	0	12	23	0	.....
	9	26	12	0	18	24	0	.....
T. sepium (71876–S)	4	11	9	6	10	19	10–15	.....
	6	18	11	11	18	35	26	A
T. serialis (94023)	6	15	5	0	13	13	7	.....
	9	25	0	0	14	26	17	A
DERMATOPHYTES								
Epidermophyton inguinale (RB)	10	6–10	10	0	12–14	3	0	.....
	14	15	18	0	18	26	0	.....
Microsporum canis (650–ATC)	7	17	17	0	21	0	0	.....
	10	26	17	0	31	0	0	.....
Trichophyton purpureum (8531–ATC)	10	12	0	0	13	5	0	.....
	14	15	2(p)	0	17	15(p)	0	.....
T. purpureum (RB)	10	10	2	0	11–12	9	0	.....
	14	10	6	0	13	10–17	0	.....

those fungi which had been found by the streak method to be most active. Forty-four of the fungi in table I were chosen for further experimentation chiefly because of their activity against *Staph. aureus* on thiamine-peptone agar and of their failure to produce extreme acidity. We included also *Ceratostomella ulmi, C. rostrocylindrica, Epicoccum* sp., and *Stereum murrayi* (71162).

A modification of a method described by Raper, Alexander and Coghill (3) was used for this group. It consisted in growing the fungus on an agar medium in Petri dishes. At intervals after inoculation discs of agar were cut on a radius extending from the center of the colony to the edge of the dish. The discs were transferred to another agar plate seeded with the test organism. The diameter of the zone of inhibition produced by antibiotic material diffusing from the disc was measured. We used 20 ml. of various agar media[7] in a Petri dish, 15×100 mm., for cultivating the fungus which was inoculated in the center of the dish and incubated at 25° C. A cork borer 5.5 mm. in diameter was used for cutting the discs. Eighteen ml. of a yeast-peptone medium were poured into a Petri dish and allowed to stand overnight. This was then coated with 7 ml. of the same medium to which were added 0.2 ml. of a 24-hour broth culture of the test bacteria diluted in the proportion of 1.2 ml. of the culture to 10 ml. of sterile beef broth. When the coating layer had solidified the discs were transferred and the plates incubated upside down at $37 \pm 2°$ C. Evidence of antibiotic activity could be observed after 5 or 6 hours, but final observations were made after 24 hours. The growth of both *Staph. aureus* and *E. coli* was considerably heavier on the yeast-peptone agar than on the thiamine-peptone agar.

Results with the Disc Method. The activity as determined by the disc method was classified as none (0), little (+), minor (++), medium (+++), and strong (++++). The strongest activity was not so great as that found for *Penicillium notatum* (P832C).

When grown at 25° C on thiamine-peptone agar little or no activity was found for *Ceratostomella ulmi, C. rostrocylindrica, Claudopus nidulans* (71364–S), *Coniophora suffocata* (56488–S), *Corticium lividum* (5159), *Flammula alnicola* (2071), *Fomes ulmarius* (CBS), *Hydnum scheidermayeri* (71787–R), *Hymenochaete corrugata* (71759–R), *H. rubiginosa* (58557–R), *Polyporus tulipiferus* (71938), *P. vulpinus* (2279), *Poria incrassata* (59125–

[7] These included the thiamine-peptone agar; a yeast-peptone medium which contained per liter 5.0 g. neopeptone, 3.0 g. yeast extract, 1.0 g. dextrose, and 15 g. Difco agar (the pH of this medium was 6.6); modified Dox plus corn steep water solids (C. M. Armstrong, Inc.) which contained per liter 3 g. $NaNO_3$, 1 g. KH_2PO_4, 0.5 g. KCl, 0.5 g. $MgSO_4 \cdot 7H_2O$, 0.01 g. $Fe_2(SO_4)_3$, 40 g. dextrose, 5 g. corn steep water solids, and 15 g. Difco agar; starch-tryptone agar which contained per liter 10 g. soluble starch, 5 g. tryptone, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, the mineral supplements previously mentioned, and a mixture of pure B complex vitamins.

R), *P. subacida* (B), and *Stereum murrayi* (71162), and only minor activity for *Fomes annosus* (58548–S), *Polyporus albellus* (14554–S), *P. resinosus* (52043–S), *P. schweinitzii* (71356–S), *Poria ferruginea-fusca* (1411), *P. sequoiae* (F–8755), and *Stereum sulcatum* (71329–R). These fungi were not grown on other media for test by the disc method. *Fomes juniperinus* (72028–S and CBS) and *F. ribis* (94142) showed little or no activity when grown on the thiamine-peptone medium, the Dox medium supplemented with

TABLE 2. *Degree of antibiotic activity in the agar disc method with the following media: thiamine-peptone agar, modified Dox plus corn steep water solids, and yeast-peptone agar*

Degrees of activity: 0 = none; + = little; ++ = minor; +++ = medium; ++++ = strong.

Fungus	Degree of activity on		
	Thiamine-peptone	Dox medium plus corn steep solids	Yeast-peptone
Corticium abeuns ? (14572–R)	++++	++++	0
C. hydnans (57407–S)	+++	+++	0
Epicoccum sp.	+++	.....	.....
Hydnum abietis ? (3548)	+++	.....	.....
H. septentrionale (52037–S)	++++	++++	.....
Irpex mollis (71542–R)	++++	++++	.....
Lepiota naucina (312)	+	+++	0
Phlebia strigosozonata (56454–S)	+++	+++	0
Pleurotus griseus ? (14616–R)	+++	++++	0
Polyporus biformis (71423–R)	++++	++++	.....
P. delectans (71089)	++++	++++	.....
P. giganteus (50382–R)	++	0	0
P. giganteus (57026)	+++	.....	.....
P. obtusus (71791–S)	++++	++++	.....
P. radiatus (B)	+++	.....	.....
P. trabeus (55196–R)	++	++	.....
Poria corticola ? (71280–R)	+	++++	+++
P. subacida (71155)	++++	++++	++
P. tenuis (67942)	++++	++++	++++
Radulum orbiculare (57402–S)	+++	+	0
Stereum gausapatum (58581)	++	+++	+
S. rugosum (86351)	0	++	0
Trametes rubescens (H–61)	++	+	0

corn steep water solids, and on the yeast-peptone medium. The results with the remaining 23 of the 48 fungi tested by the disc method are given in table 2.

In several instances the correlation between the results obtained with the streak method and the disc method was poor, i.e., considerable inhibition was observed with the streak method but no, or little, activity with the disc method of investigation. It is probable that this difference occurred because the antibiotic substance diffused widely in the agar on which the fungus grew, but did not accumulate in sufficient amount to produce an effect when discs were removed and tested on a fresh medium. There are other possible expla-

nations, some of which were examined for *Fomes ribis* (94142), one of the organisms which evidenced considerable activity against *Staph. aureus* by the streak method but none when tested by the disc method (fig. 1, A and B). The streak tests were carried out on the thiamine-peptone agar, the medium

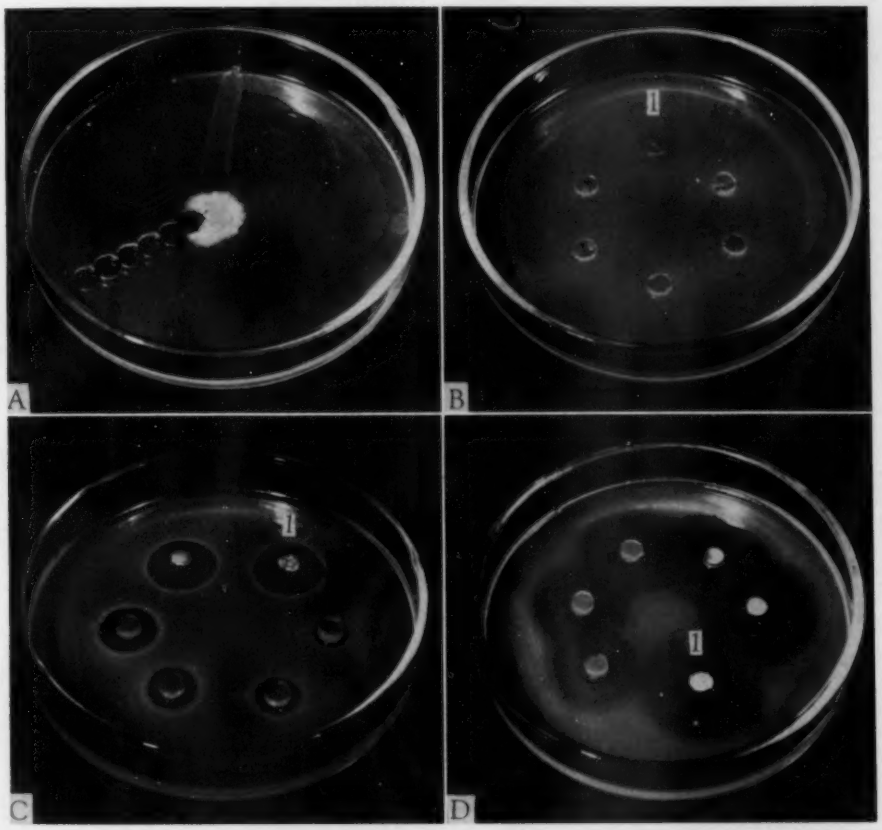

FIG. 1. A, antibiotic activity of 7-day-old *Fomes ribis* (94142) tested by streak method on thiamine-peptone agar. *Staph. aureus* largely inhibited, *E. coli* not affected. B, tested by disc method, no antibiotic activity against *Staph. aureus*. C, complete inhibition of *Staph. aureus* with stimulation of bacteria at edge of inhibited zone. Discs from colony of *Pleurotus griseus* (14616–R) grown 17 days on modified Dox medium plus corn steep water solids. D, partial inhibition of *Staph. aureus*. Discs from *Poria tenuis* (67942) grown 14 days on modified Dox medium plus corn steep water solids. The number, 1, marks the disc taken nearest the center of the colony.

on which the fungus grew; the discs were tested on the yeast-peptone medium. However, we found little or no activity for the discs from a culture of *Fomes ribis* when tested on the thiamine-peptone medium. We found further that the difference between the streak method and the disc method for

this fungus was not the result of slow diffusion of the antibiotic substance from the disc, or to a lack of stability of the antibiotic substance at $37 \pm 2°$ C.

The medium on which the fungus was grown markedly affected the formation of antibiotic material. For example, we observed little antibiotic activity of *Penicillium notatum* grown on thiamine-peptone agar, of *Poria corticola* (71280–R) on a starch-tryptone or thiamine-peptone medium, of

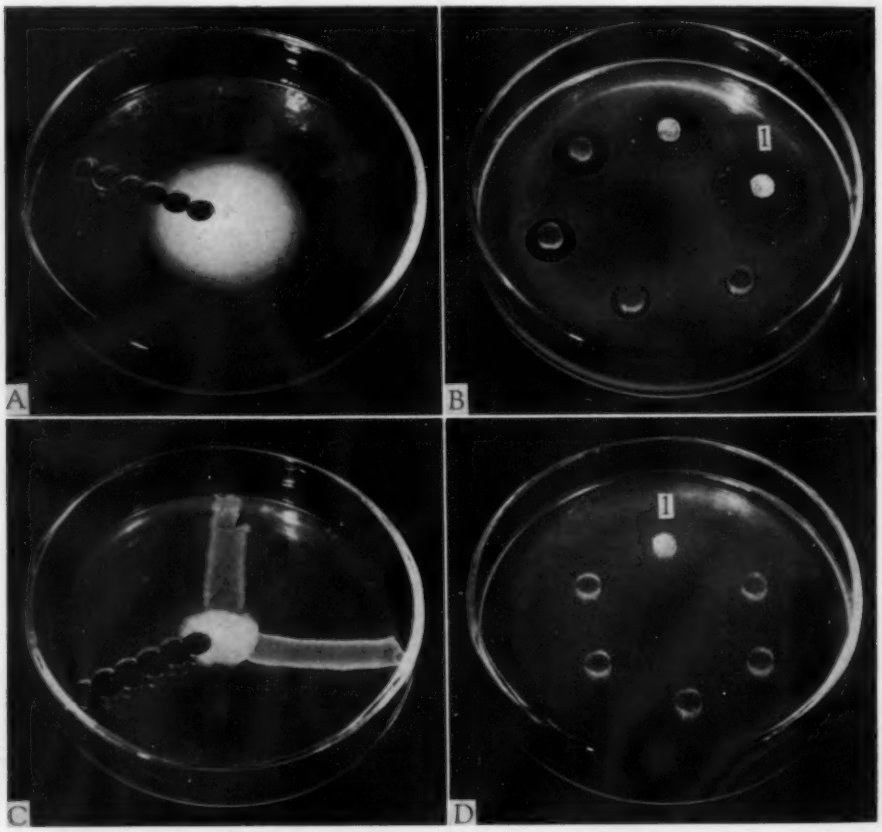

FIG. 2. Effect of medium on production of antibacterial material. A, *Corticium abeuns* (14572–R), 17 days old, on Dox medium plus corn steep water solids. *Staph. aureus* completely inhibited, *E. coli* partially. B, discs from A tested against *Staph. aureus*. Complete inhibition with resistant colonies. C and D, same as A and B, but fungus grown on yeast-peptone agar. No inhibition of streaks and no antibiotic action with discs. The number, 1, marks the disc taken nearest the center of the colony.

Corticium abeuns (14572–R) (fig. 2) and *Pleurotus griseus* (14616–R) on a yeast-peptone medium. In general, the modified Dox medium plus corn steep water solids was the most satisfactory medium for the production of antibacterial substances by these fungi (table 2).

It was not possible to predict from the results obtained with one fungus the effect of a particular medium on the antibiotic activity of another mold. For example, the yeast-peptone medium was quite satisfactory for *Poria tenuis* (67942) and for *Penicillium notatum;* it was poor for *Poria subacida* (71155), and no antibiotic activity of *Corticium abeuns* (14572–R) and *Pleurotus griseus* (14616–R) was observed on this medium when the disc method was used.

Two types of antibiotic action were observed as follows:

(1) The antibiotic material diffusing out from the disc completely inhibited the bacteria; the disc was surrounded by a sharply defined clear ring. In some instances the growth of the bacteria at the edge of the ring appeared to be stimulated (fig. 1, C). This type of inhibition appeared to be bactericidal in character and is referred to as "complete."

(2) The antibiotic material held back the growth of the bacteria in the vicinity of the disc but did not eliminate it completely; the area of inhibition appeared cloudy because of the presence of numerous small bacterial colonies (fig. 1, D). This type of inhibition is called "partial," and was bacteriostatic rather than bactericidal. Frequently with this type the disc was surrounded by zones of inhibition alternating with zones in which the inhibition was less marked. This gave the area of bacteriostatic activity the appearance of a bull's-eye or of Liesegang rings.

Between these two clearly marked types various intermediates were observed. For example, we found in some instances a narrow zone of complete inhibition surrounded by a broader zone of partial inhibition, and a type in which the area of inhibition contained a considerable but reduced number of colonies of somewhat larger size than those which developed in the balance of the plate.

These differences in types of antibiotic action indicate differences in the kind or in the amount of antibiotic and perhaps stimulating substances produced by the various fungi.

For some of the fungi the antibiotic activity of discs taken from within the limits of the mycelium decreased with time; the disc with greatest antibiotic activity was that at or near the edge of the colony. This occurred with *Poria corticola* (71280) and *Poria subacida* (71155) (fig. 3, B). For other fungi the antibiotic activity persisted and increased within the mycelium; the disc with greatest antibiotic activity was that nearest the center of the fungus colony. This was especially marked with *Corticium abeuns* (14572–R) (fig. 3, A and fig. 2, B) and *Poria tenuis* (67942) (fig. 3, D).

The antibiotic activity did not increase with increased rapidity of growth when a fungus was grown on different media on which rates of growth differed. It was not greater with those species of fungi which grew more rapidly as compared with those which grew less rapidly. In fact, it appeared in many

instances that the correlation between growth and antibiotic activity, as measured by the disc method, was negative. Many of the fungi which grew rapidly showed little or no antibiotic activity (or the action could be accounted for by acid production), and a particular fungus might evidence little antibiotic activity on a medium on which it grew well and considerable on one on which it grew poorly (fig. 4).

The antibiotic material from some of the fungi tested diffused beyond the limits of the mycelium; discs 1.5 or 2.0 mm. from the edge of the colony

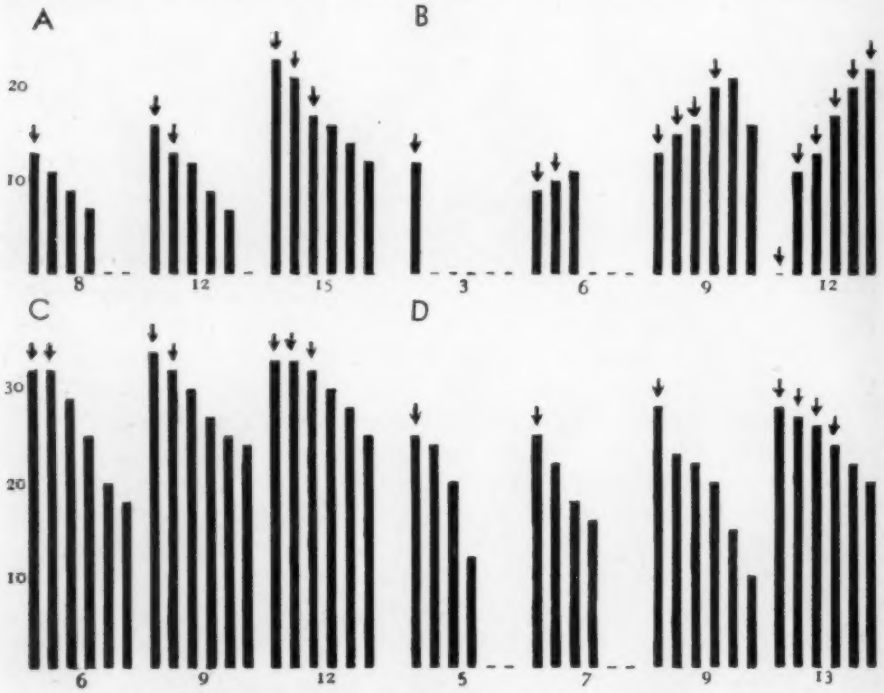

FIG. 3. Diameter in mm. of inhibition zones for *Staph. aureus* produced by discs from various fungi. Each test includes 6 discs taken from center of colony outward. All fungi grown on Dox medium plus corn steep water solids. The arrows show those discs which came from the fungus colony. A, *Corticium abeuns* (14572–R), 8, 12, 15 days old; B, *Poria subacida* (71155), 3, 6, 9 and 12 days old; C, *Penicillium notatum* (P832C1), 6, 9 and 12 days old; D, *Poria tenuis* (67942), 5, 7, 9 and 13 days old.

were active (fig. 3, D). With other fungi the only active discs were those removed from within the colony. For example, discs from the colony of *Hydnum septentrionale* (52037–S) were strongly active, producing zones of complete inhibition with a diameter of as much as 24 mm. However, discs taken from outside the limits of the colony were inactive. The reasons for this are not clear. It may indicate that the antibiotic material is largely retained by the mycelium and not freed until the discs are cut and incubated at

$37 \pm 2°$ C. We were not successful in releasing the material by incubating uninjured colonies of the fungus at the higher temperature.

The observations on the more active fungi tabulated in table 2 were as follows:

Corticium abeuns (14572–R) grew with medium rapidity; the inhibition

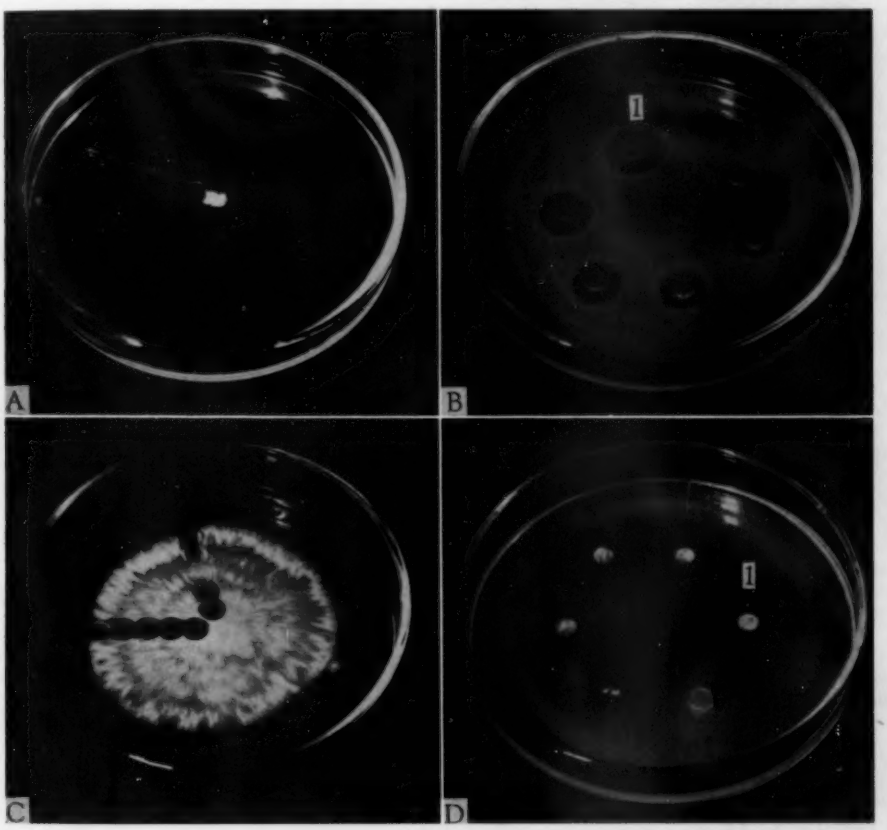

FIG. 4. An extreme example of negative correlation between growth and antibiotic activity. *Pleurotus griseus* (14616–R) grown: A, 21 days on a thiamine-peptone agar with 100 g. dextrose per liter; B, discs from A on plate seeded with *Staph. aureus;* C, 19 days on Dox medium plus corn steep water solids and 20 g. brown sugar per liter; D, discs from C on plate seeded with *Staph. aureus.* The number, 1, marks the disc taken from nearest the center of the colony.

was complete with scattered resistant colonies; the antibiotic material diffused widely and persisted within the colony (fig. 2, B).

C. hydnans (57407–S) resembled *C. abeuns* but was less active.

Epicoccum sp. grew rapidly; the inhibition was complete; the antibiotic material did not diffuse much beyond the limits of the colony and decreased within the colony.

Hydnum abietis? (3548) grew slowly; the inhibition was complete; the antibiotic material diffused widely and decreased with time within the colony.

H. septentrionale (52037–S) grew with medium rapidity; the inhibition was complete; the antibiotic material did not diffuse beyond the colony and persisted within the colony.

Irpex mollis (71542–R) grew rapidly; the inhibition was complete becoming partial with age on the thiamine-peptone medium; the antibiotic material diffused widely and persisted well within the colony.

Lepiota naucina (312) grew with medium rapidity; the inhibition was complete; the antibiotic material was limited to the colony and persisted well within the colony.

Phlebia strigosozonata (56454–S) grew slowly; the inhibition was complete; the antibiotic material diffused beyond the colony and persisted well within the colony.

Pleurotus griseus (14616–R) grew with medium rapidity; the inhibition was complete; the antibiotic material diffused widely and persisted within the colony (fig. 1, C).

Polyporus biformis (71423–R) grew rapidly; the inhibition was variable in type; the antibiotic material was largely limited to the colony and tended to decrease within the colony with time.

P. delectans (71089) grew rapidly; the inhibition was complete; the antibiotic material was largely confined to the colony and persisted well within the colony.

P. obtusus (71791–S) resembled *P. delectans* (71089).

P. radiatus (B) resembled *Corticium hydnans.*

Poria corticola? (71280–R) grew rapidly; the inhibition was largely partial; the antibiotic material diffused widely and decreased within the colony with age.

P. tenuis (67942) grew with medium rapidity; the inhibition was mainly partial; the antibiotic material diffused widely and persisted well within the colony (fig. 1, D).

P. subacida (71155) grew rapidly; the inhibition was mainly partial; the antibiotic material was mainly confined to the colony and decreased within the colony with age (fig. 3, B).

Radulum orbiculare (57402–S) grew slowly; the inhibition was complete; the antibiotic material diffused widely and persisted well within the colony.

Stereum gausapatum (58581) grew with medium rapidity; the inhibition was complete; the antibiotic material diffused widely and persisted within the colony.

Serial Dilution Method. Although the disc method has certain advantages, its usefulness is limited to cultures in which the fungus is grown on

a semisolid medium. If it is desired to test the antibiotic agent on animals, or to concentrate and perhaps isolate it, culture liquids are necessary unless the agent can be extracted from the mycelium. A convenient method of testing the activity of culture liquids is to determine the action of a series of dilutions on the growth of the test bacterium. Our procedure was as follows:

The fungus was grown in flasks at 25° C until a mycelial mat had formed over the surface of the culture liquid. At various intervals after the mat had formed the old culture liquid was poured off and replaced with fresh culture fluid, the antibiotic activity of which was tested after 1, 3, and 5 days contact with the mycelial mat. For some mats this process was repeated several times.

For the serial dilutions 1 ml. of a 6-hour broth culture of *Staph. aureus*, which had been diluted 1 to 100,000 with beef broth, was placed in standard tubes, 10 × 75 mm. To the first tube 1 ml. of the culture fluid (neutralized if necessary) was added, giving a dilution of 1 to 2. One ml. from this tube was transferred to the second tube, giving a dilution of 1 to 4. This was continued for a set of 10 or more tubes. The tubes were incubated for 24 hours at 37 ± 2° C and examined for bacterial growth.

Results with Serial Dilutions. With a few exceptions the antibiotic activity of culture liquids from the fungi we examined was unsatisfactory. For *Corticium abeuns* (14572–R), *Fomes juniperinus* (CBS and 72028–S), *Fomes ribis* (94142), *Lepiota naucina* (312), *Polyporus giganteus* (50382–R and 57026), *Poria ferruginia-fusca* (1411), *P. subacida* (71155), *Radulum orbiculare* (57402), *Stereum rugosum* (86351), *S. sulcatum* (71329–R), and *Trametes rubescens* (H61) the maximum activity did not surpass dilutions of 1 to 64, and in many instances was less. With *Poria corticola* (71280–R) and *P. tenuis* (67942) culture fluids active at a dilution of 1 to 256, and for *Pleurotus griseus* (14616–R) and *Irpex mollis* (71542–R) culture liquids active at a dilution of 1 to 1024 were obtained. Mats of *Pleurotus griseus* which were 50 days old were still effective in producing antibiotic material.

DISCUSSION

A considerable proportion of the wood-destroying fungi included in this survey showed antibiotic activity, and for most of these it appeared to be caused by other substances than the hydrogen-ion. Antibiotic activity was not confined to particular genera. One or more species of 43 of the 72 genera included in this survey showed some antibiotic activity. Several of the genera which showed no activity were represented in our survey by a single species. Of the 94 species and isolations of *Polyporus* studied by the streak method, 51 were inactive, 19 gave inhibitions of 10 mm. or less, and 24 of 11 mm. or more. Of the 24, we considered the antibiotic activity of 11 to be caused by acid production. Of the 47 *Fomes* surveyed, 15 gave negative results; 13,

inhibitions of 10 mm. or less; and 19, of 11 mm. or more. The antibiotic activity of 7 of the 19 was probably the result of acid production. Of 41 *Porias* studied, 9 were inactive and 19 produced inhibitions of 11 mm. or more. The antibiotic activity of 11 of the 19 was probably caused by acid formed by the fungi.

In many instances the different isolations of a species were alike in antibiotic activity. For example, 2 isolations of *Polyporus anceps* were both negative, two isolations of *P. circinatus* were slightly antibiotic. On the other hand, although the two isolations we tested of *P. delectans* were alike morphologically, one was negative and one was strongly antibiotic.

However, in some respects the results of the investigation were disappointing. None of the more than 400 fungi investigated was as effective as *Penicillium notatum* in producing material active against *Staph. aureus*, and although some inhibited *E. coli*, none affected the gram-negative bacterium as much as the gram-positive, except in those instances in which the inhibition appeared to be caused by acidity.

It might be recalled, however, that the culture liquids of *Penicillium notatum* with which Fleming initiated his investigations of that organism were effective against *Staph. aureus* at a dilution of 1 to 800 (1). By modifying the cultural conditions and the composition of the culture liquid it has been possible to increase this activity several times. In our experiments culture liquids were obtained from *Pleurotus griseus* which were active at a dilution of 1 to 1000 and from *Poria corticola* and *Poria tenuis* active at a dilution of 1 to 250. Whether the activity of these fungi also can be materially increased by selection of strains, or by modification of cultural conditions, can only be determined by further research. In addition, we have not in this survey investigated the possibility that antibacterial agents may be present in the mycelium and not released into the culture fluid.

Some miscellaneous observations were made in the course of our survey which suggest that the antibiotic agents produced by some of these fungi are not penicillin, and that the substances formed by some, at least, of the fungi studied are not identical. For example, the character of the area of inhibition around the blocks differed. *Corticium abeuns* produced complete inhibition with many resistant colonies toward the edge of the clear area. *Pleurotus griseus* completely inhibited *Staph. aureus*, but the resistant colonies so characteristic for *Corticium abeuns* were absent. For both of these fungi the edge of the clear area was sharply defined and lacked the transition zone of partially inhibited colonies noted with *Penicillium notatum*. *Poria corticola* (71280–R), *P. subacida* (71155), and *P. tenuis* (67942) all showed partial inhibition clearly distinguishable from the action of *Penicillium notatum*, *Corticium abeuns*, and *Pleurotus griseus*. Of the three species of *Poria* the activity of *P. tenuis* could be distinguished from that of the other

two by the persistence of antibiotic activity within the colony. From these six fungi there appeared to be at least four and perhaps five different antibiotic agents. Of course at the present stage of our investigations we have no evidence on whether the activity of any one of the wood-destroying fungi results from one or more than one antibiotic agent.

The antibiotic material produced by *Corticium abeuns* was distinguished from penicillin and streptothricin by testing against penicillin and streptothricin a strain of *Staph. aureus* resistant to the antibiotic material produced by this fungus. Strains of *Staph. aureus* resistant to the action of *Corticium abeuns* were isolated from resistant colonies in the vicinity of discs taken from the fungus (fig. 2, B). The bacterium resistant to the antibiotic culture fluid from *Corticium abeuns* was as susceptible to penicillin as the normal strain of *Staph. aureus* and nearly as susceptible to streptothricin as the normal strain.

Whether any of the fungi included in this survey are possible sources of important antibiotic materials must be determined by further investigation.

SUMMARY

The antibacterial activity of over 400 fungi including over 300 wood-destroying organisms and 22 dermatophytes against *Staphylococcus aureus* and *Escherichia coli* was surveyed. Somewhat over 200 of the fungi exhibited antibacterial activity. For a considerable proportion the inhibition of bacterial growth did not appear to be caused by hydrogen-ion concentration developed by the fungus. None was as effective as *Penicillium notatum* in producing material active against *Staph. aureus*, and although some inhibited *E. coli* none affected the latter organism as much as it affected *Staph. aureus*. Culture liquids active against *Staph. aureus* at a dilution of 1 to 1000 were obtained from *Pleurotus griseus* and *Irpex mollis*.

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STUDIES IN THE SAPOTACEAE—I. THE NORTH AMERICAN SPECIES OF CHRYSOPHYLLUM

ARTHUR CRONQUIST

For the purpose of this series of papers, North America is taken to include Mexico, Central America, and the West Indies, exclusive of Trinidad and Tobago. The work is done under the auspices of the Chicle Development Co., under the direction of Mr. B. A. Krukoff. I extend my thanks to them and to the curators of the following herbaria (designated hereinafter by the letters at the left), who have kindly loaned specimens for study:

A, Arnold Arboretum, Harvard University, Jamaica Plain, Massachusetts.

CR, Museo Nacional de Costa Rica, San José.

F, Chicago Natural History Museum, Chicago, Illinois.

G, Gray Herbarium, Harvard University, Cambridge, Massachusetts.

Mich, University of Michigan, Ann Arbor, Michigan.

MO, Missouri Botanical Garden, St. Louis, Missouri.

NY, New York Botanical Garden, New York.

PR, Tropical Forest Herbarium, U. S. Forest Service, Rio Piedras, Puerto Rico.

US, United States National Herbarium, Washington, D. C.

Y, Yale University School of Forestry, New Haven, Connecticut.

Since it is at present impossible to verify many of the older names, current interpretations of their application are accepted. No attempt is made to give full synonymy for the period before 1900. This can generally be found elsewhere, as in Pierre and Urban's treatment of the Sapotaceae of the West Indies (Symb. Ant. **5**: 95–176. 1904).

In North America, at least, the genus *Chrysophyllum* is sufficiently well characterized and sharply enough separated from the other genera of the family so that it becomes feasible to treat it separately. The following generic description is intended only for North American plants, although it will of course apply in large part to the genus as a whole.

CHRYSOPHYLLUM L. Sp. Pl. 192. 1753

Shrubs or small to medium-sized trees with alternate exstipulate leaves; secondary lateral veins generally nearly parallel to the primary ones, the areolae with the long axis about parallel to the primary veins; flowers few to numerous in axillary clusters, occasionally solitary; sepals 5, occasionally 4 or 6, joined only at the base, not over 3 mm. long; corolla not over 6 mm. long, cylindrical or campanulate, with 5 (occasionally 4 or 6) lobes; stamens

as many as the corolla-lobes and opposite them; filaments inserted at or near the level of the sinuses, often connected by a slightly thickened ring in the corolla-throat; anthers extrorse, but the valves often so twisted as to open subintrorsely; staminodia absent, or occasionally 1 or more irregularly developed in the sinuses, in individual flowers; ovary about 4–12-celled, bearing a short columnar style and a single capitate more or less lobed stigma; ovules solitary in the locules, attached laterally or basilaterally, in flower; fruit fleshy, with one or sometimes several seeds; seed-scar large, broadly elliptic to subcordate, generally at least 5 mm. long, lateral or basilateral; endosperm copious.

Type species: *Chrysophyllum cainito* L.

KEY TO THE SPECIES

1. Leaves loosely spreading-hirsutulous beneath, the hairs strongly rufous when young, later sometimes fading, and perhaps eventually deciduous; calyx 2–3 mm. long;[1] immature fruit ellipsoid, over 2.5 cm. long; Costa Rica. 8. *C. hirsutum.*

1. Leaves from permanently rufous-tomentose to finely white-sericeous or strigose and glabrate beneath, but not loosely hirsutulous; calyx various, sometimes but not often over 2 mm. long; fruit various.

2. Leaves densely and permanently rufous-tomentose or -villous beneath, sometimes becoming paler in age.

3. Stigma-lobes mostly 7–12; fruit several-seeded, 3 cm. thick or more, generally subglobose; general distribution ..... 6. *C. cainito.*

3. Stigma-lobes mostly 5, rarely 4 or 6; fruit 1-seeded, much less than 3 cm. thick, subglobose or more commonly ellipsoid.

4. Fruit subglobose, less than 1 cm. thick; leaves more or less obovate-elliptic, broadest above the middle, obtuse at the apex; Cuba ..... 9. *C. claraense.*

4. Fruit ellipsoid, much longer than thick; leaves variously elliptic to ovate or lanceolate, or rarely obovate, generally broadest at or below the middle, often but not always pointed at the tip.

5. Flowers appearing on old defoliated branches at the time new leaves are appearing elsewhere; fruit about 3 cm. long and 2 cm. thick; Tepic, Mexico ..... 7. *C. tepicense.*

5. Flowers generally appearing in the axils of leaves; fruit not over 2.5 cm. long, about 1 cm. thick or less.

6. Leaves narrowly elliptic or elliptic-lanceolate, acuminate at both ends; Haiti and the Dominican Republic ..... 12. *C. angustifolium.*

6. Leaves evidently broader, generally rounded to acutish at the base.

7. Corolla 3.1–5.0 mm. long, the lobes distinctly shorter than the tube, as measured from the inside, generally not at all auriculate; filaments inserted about at the level of the sinuses, connected by a slightly thickened ring in the corolla-throat; Greater Antilles, Bahama Islands, and Florida ..... 10. *C. oliviforme.*

[1] Measurements of the calyx are taken from the inside, where it is sharply differentiated from the receptacle.

7. Corolla 2.2–3.5 mm. long, the lobes equaling or generally a little exceeding the tube, as measured from the inside, commonly slightly auriculate; filaments generally inserted on the corolla-lobes, a little above the level of the sinuses, the thickened ring absent or obscure; Tamaulipas and San Luis Potosi, Mexico, to Honduras and El Salvador ..... 11. *C. mexicanum.*

2. Leaves finely appressed-villosulous or strigulose beneath, with usually white hairs, when young, sooner or later glabrate (sometimes more densely hairy and somewhat rufous in *C. bicolor*, but still glabrate).

3. Creases for the corolla-lobes showing plainly on the outside for about the same length as the free part of the lobes, because the lobes are joined within by a thin cylinder of tissue; calyx 1.0–1.5 mm. long; fruit only slightly if at all longer than thick, usually several-seeded; Panama and Costa Rica ..... 4. *C. panamense.*

3. Creases for the corolla-lobes extending only slightly if at all beyond the free part of the lobes; calyx often more than 1.5 mm. long; fruit various, often much longer than wide, generally single-seeded except in *C. bicolor.*

4. Filament-traces, or some of them, evident.

5. Corolla finely sericeous to near the base, the hairs white or somewhat yellowish, not rufous; stigma-lobes 5 or often more, sometimes as many as 9; fruit rounded at the end, not tapering to the style; general throughout the West Indies ..... 1. *C. argenteum.*

5. Corolla sparsely or moderately rufous-hairy above the middle, the hairs much coarser than in *C. argenteum*, generally confined to 5 patches, one on the lower part of each lobe and extending to the upper part of the tube; stigma-lobes 5; fruit generally tapering to the style; Puerto Rico and the Virgin Islands ..... 2. *C. pauciflorum.*

4. Filament-traces obscure.

5. Fruit nearly globose, about 1.5–2.0 cm. thick; stigma-lobes usually but not always more than 5; pubescence of the pedicels strongly rufous; Puerto Rico and the Virgin Islands ..... 5. *C. bicolor.*

5. Fruit ellipsoid, about 1 cm. thick or less; stigma-lobes 5, or occasionally 4 or 6; pubescence of the pedicels more or less grayish, or absent; Mexico and Central America.

6. Corolla more or less hairy on the outside, about 3.0–4.0 mm. long; Costa Rica ..... 3. *C. brenesii.*

6. Corolla glabrous, 2.2–3.5 mm. long; Mexico to Honduras. 11. *C. mexicanum.*

1. CHRYSOPHYLLUM ARGENTEUM Jacq. Enum. Pl. Carib. 15. 1760.

C. glabrum Jacq. Enum. Pl. Carib. 15. 1760.
C. caeruleum Jacq. Stirp. Select. Am. 52. ca. 1780.
C. argenteum var. *sphaerocarpum* Urb. Symb. Ant. 9: 417. 1925.

Commonly a small tree, sometimes larger and as much as 30 m. high, or sometimes a mere large shrub 3–4 m. high; leaves glabrous above, thinly sericeous or strigulose with whitish hairs beneath, sooner or later glabrate, commonly elliptic or oblong-elliptic, broadest near the middle, but carrying the width well toward both ends, sometimes more ovate, of various sizes up to about 20 cm. long and 8 cm. wide, rounded to acutish or abruptly acumi-

nate at the apex, the very tip blunt; flowers generally several or numerous in a cluster, sometimes only a few, the pedicels mostly 4–13 mm. long; calyx about 1.5–2.5 mm. long, the sepals broad, rounded, more or less sericeous outside, with fine light-colored or slightly reddish hairs, sometimes also slightly hairy inside, the margins usually thin and smooth; corolla white to greenish or yellow, 4–6 mm. long, sericeous to near the base with fine white or somewhat yellowish hairs, the tips and margins of the lobes smooth, the lobes generally about half as long as the tube, or a little less, broadly rounded; filament-traces more or less conspicuous, or sometimes some of them obscure; anthers 0.4–0.8 mm. long; stigma 5–9 lobed, the lobes evident to obscure; fruit 1-seeded, dark red or purple to blue or nearly black, commonly ellipsoid, often rather broadly so, about 1.5–2 cm. long, varying to subglobose and only 1 cm. long; seed-scar basilateral, commonly extending to near the middle.

TYPE: None given; from Martinique, according to Index Kewensis.

DISTRIBUTION: Forests; general throughout the West Indies from Oriente, Cuba, to Trinidad; apparently not on the mainland; reported by collectors to vary from fairly common to rare.

LOCAL NAMES: caimitillo, petit caymite, carabam, lechesillo, caimito verde, bois-rabi, kaimitier-bois, bois de bouis, acomat, bois-glu, bois-bouis, petit bois, petit bouis, buit, buis, bris, buie, wild star apple.

CUBA: *Crosby & Matthews s.n.* (Y); *Eggers 4759* (NY); *Leon 11780* (US); Matanzas: *?Rugel 627* (G); Oriente: *Ekman 4076* (US); *Ekman 4142* (NY); *Ekman 7376* (NY); *Shafer 4419* (NY); *Shafer 7709* (G, NY); *Shafer 8815* (G, NY, US); *Wright 345* (G, NY); *Wright 1328* (G, MO); *Wright 1636* (G, MO, NY). HAITI: *Ekman 1464* (US); *Ekman 2344* (US); *Ekman 2387* (US); *Leonard 5291* (NY, US); *Leonard 8675* (G, NY, US); *Leonard & Leonard 12423* (G, US); *Leonard & Leonard 12445* (MO, US); *Leonard & Leonard 12945* (A, NY, US); *Leonard & Leonard 14519* (Mich, US); *Nash 192* (NY); *Nash 465* (NY); *Nash 606* (NY); *Nash & Taylor 1205* (NY). DOMINICAN REPUBLIC: *Abbot 1526* (G, US); *Abbot 1604* (G, US); *Abbot 2159* (G, US); *Eggers 2433* (A, NY, US); *Ekman 15757* (US); *Ekman 16442* (US); *Fuertes 446* (A, G, MO, NY, US); *Taylor 189* (NY); *Taylor 299* (NY); *Rose, Fitch & Russell 4332* (NY, US); *Wright, Parry & Brummel 202* (US). PUERTO RICO: *Britton & Britton 7201* (NY); *Britton & Britton 7422* (NY, US); *Britton & Britton 9980* (NY); *Britton, Britton & Brown 6205* (NY); *Britton, Cowell & Brown 4531* (NY); *Britton, Cowell & Brown 5588* (G, MO, NY, US); *Britton & Marble 955* (NY, US); *Eggers 420* (G); *Eggers 614* (NY); *Eggers s.n.* (June 1887) (US); *Gregory 52* (NY, PR); *Heller 6431* (NY, US); *Hioram s.n.* (May, 1914) (NY, US); *Holdridge 396* (PR); *Johnston 579* (NY); *Otero 699* (NY); *Otero 702* (NY); *Shafer 3538* (NY, US); *Sintenis 1162* (US); *Sintenis 1465* (G, NY, US); *Sintenis 1937* (MO, NY, US); *Sintenis 4090* (US); *Sintenis 4130* (G, NY, US); *Sintenis 6205* (US); *Sintenis 6234* (US); *Stevenson 6310* (US); *Underwood & Griggs 886* (NY, US); *Wydler 317* (F). ST. THOMAS: *Britton & Marble 1323* (NY, US); *Eggers s.n.* (Sept. 22, 1876) (MO). TORTOLA: *Britton & Shafer 824* (NY, US); *Fishlock 478* (NY, US). SABA: *Boldingh 2115* (NY). ANTIGUA: *Box 861* (A, US); *Box 1053* (US); *Box 1346* (US). GUADELOUPE: *Bertero s.n.* (MO); *Duss 2911 (3906)* (NY, MO, F, G, A); *Duss 2912* (F, G, MO, US, NY); *Questel 1674* (US); *Questel 2264* (US); *Stehle 478* (US, Mich); *Stehle 997* (NY). DOMINICA: *Cooper 181* (G, NY, US); *Hodge 1955* (G); *Hodge 3368* (G); *Ramage s.n.* (August 23, 1888) (NY); *Ramage s.n.* (Feb. 4, 1889) (G). MARTINIQUE: *Duss 263* (NY); *Duss 265* (=*266,269*) (A, F, G, MO, NY, US); *Duss 747* (NY); *Hahn 717* (=*641*) (NY); *Hahn s.n.* (July, 1869) (G, US); *Sieber 71* (MO); *Stehle 999* (NY); *Stehle 2345* (NY); *Stehle 5188* (US). ST. VINCENT: *Eggers 6584* (A); *H. H. & G. W. Smith 912* (G); *H. H. & G. W. Smith 1283* (NY); *H. H. & G. W. Smith 1756* (G); *H. H. & G. W. Smith s.n.* (Sept. 1889) (NY). GRENADA: *Broadway s.n.* (Feb. 14, 1906) (F, G); *Eggers 6152* (A, US); *Eggers 6474* (A, US).

2. CHRYSOPHYLLUM PAUCIFLORUM Lam. Tab. Encyc. **2**: 44. 1793.

C. pauciflorum var. *nervosum* Pierre, Symb. Ant. **5**: 159. 1904.
C. pauciflorum var. *krugii* Pierre, l. c.

Tree mostly 4–20 m. high; leaves glabrous and shining above, thinly sericeous or strigulose beneath when young, soon glabrate, mostly lance-ovate or narrowly ovate and somewhat acuminate, sometimes more elliptic and merely acutish, of various sizes up to about 10 cm. long and 4 cm. wide, averaging much smaller than in *C. argenteum;* flowers mostly 1–5 in a cluster, the pedicels slender and often lax, about 3–10 mm. long, distinctly rufous-hairy; calyx about 1.3–2.0 mm. long, the lobes rufous-hairy, narrower than in *C. argenteum,* and, especially the outer, tending to be acutish, often becoming progressively shorter, broader, and more rounded in centripetal progression; corolla reported to be yellowish, about 3.5–5.0 mm. long, with a distinctly reddish-hairy patch near the base of each lobe and extending to the upper part of the tube, the patches rarely confluent on the tube, the hairs coarser and sparser than those of *C. argenteum;* corolla-lobes about 2/5–4/5 as long as the tube; filament-traces evident, or occasionally some of them obscure; anthers about 0.5–0.7 mm. long; stigma evidently 5-lobed; fruit 1-seeded, ellipsoid, generally rather narrowly so, somewhat tapering to the style and often also tapering to the base, commonly about 1.5 cm. long; seed more slender than in *C. argenteum,* tapering to each end; seed-scar basilateral, generally extending to about the middle.

TYPE: Lamarck cites no specimens, but refers to plate 38, figure 2, in Jacquin's Stirp. Am. Icon. 1760, and indicates Martinique as the habitat. The fruit shown there can scarcely be that of the plant currently called *C. pauciflorum,* but looks more like that of *C. argenteum.* The flowers are shown as solitary in the axils, however, as in *C. pauciflorum.* The leaves shown in the figure might belong to either species. Since the drawing shows flowers and mature fruit on the same twig, it seems not unlikely that it was synthetized from two specimens, one of each species. *C. pauciflorum,* as currently interpreted, does not occur on Martinique, but is restricted to Puerto Rico and the Virgin Islands. Alphonse De Candolle, in his treatment of *C. pauciflorum* in the Prodromus (**8**: 158. 1844.) indicates that he has seen a specimen from St. Thomas in the herbarium of the Museum of Paris. It seems probable that this specimen was available to Lamarck, and should be taken as the type, with the reference to the Jacquin drawing excluded. It is perhaps noteworthy that the specimen on which De Candolle's description was based had young fruit, but no mature fruit.

DISTRIBUTION: Wooded places; Puerto Rico and the Virgin Islands.

LOCAL NAME: Caimito de perro.

PUERTO RICO: *Britton & Britton 8847* (NY); *Britton & Britton 9306* (NY); *Britton & Britton 9470* (NY); *Britton & Britton 9800* (NY); *Britton & Britton 9912* (NY); *Britton, Britton & Brown 5903* (NY, US); *Britton & Cowell 1328* (NY, US); *Britton & Marble 2246* (NY, MO, US); *Cook K.91* (NY); *Shafer 2532* (NY, US); *Sintenis 1991* (G, MO, NY); *Stevens & Hess 4001* (NY); *Underwood & Griggs 574* (NY, US). ST. THOMAS: *Oersted 12344* (F); *Britton & Britton 244* (G, NY, US); *Britton & Marble 1373* (NY, US); *Eggers 23* (G); *Shafer & Fitch 1471* (NY, US). ST. JAN: *Britton & Shafer 206* (NY, US). ST. CROIX: *Britton, Britton & Kemp 148* (NY); *Ricksecker 180* (MO, US); *Thompson 431* (NY).

3. **Chrysophyllum brenesii** Cronquist, sp. nov.

Tree reported to be 5–10 m. high; leaves thinly white-sericeous or strigulose on both sides when young, soon glabrate and shining above, more tardily glabrate and dull beneath, elliptic, acuminate at both ends, or the base merely acute, up to 10 cm. long and 4 cm. wide; flowers several or fairly numerous in each cluster, the pedicels about 4–8 mm. long, provided with a thin covering of fine and appressed grayish hairs; calyx about 1.4–1.7 mm. long, the sepals appressed-hairy with gray hairs outside, and somewhat ciliolate on the margins, the outer sometimes also somewhat hairy within, broadly ovate and tending to be obtuse or acutish, the inner glabrous within, suborbicular and more rounded; corolla about 3–4 mm. long, with a hairy patch near the base of each lobe and extending to the top of the tube, the hairs often somewhat rufous; corolla-lobes about equaling the tube, or a little shorter; filament-traces obscure; anthers about 0.5–0.6 mm. long; stigma evidently 5-lobed; fruit (only one seen) apparently single-seeded, broadly ellipsoid or ellipsoid-obovate, 16 mm. long, 10 mm. thick, broadest a little above the middle, constricted at the apex to a short broad beak about 1.5 mm. long and 2.5 mm. thick.

Arbor 5–10 m. alta, foliis glabratis ellipticis usque ad 10 cm. longis 4 cm. latis apicibus acuminatis basibus acuminatis vel acutis, floribus paucis vel subnumerosis pedicellis cinereis pilis appressis, corolla 3.0–4.0 mm. longa lobis tubo subaequilongis, antheris ca. 0.5–0.6 mm. longis, partibus adnatis filamentorum obscuris, drupis obovato-ellipticis 16 mm. longis 10 mm. crassis apicibus constrictis.

TYPE: *Brenes 4415*, hill of San Pedro de San Ramon, Alajuela, Costa Rica, 1025 m., September 3, 1925 (F); ISOTYPE (CR).

DISTRIBUTION: San José and Alajuela, Costa Rica, at 1000–1100 m.

COSTA RICA: *Brenes 4155* (CR); *Brenes 15623* (CR). San José: *Tonduz 12718* (= Donnell-Smith Distr. 7443) (US, CR, G). Alajuela: *Brenes 5021* (F, CR); *Brenes 5446* (F, CR); *Quiros 972* (CR).

4. CHRYSOPHYLLUM PANAMENSE Pittier, Contr. U. S. Nat. Herb. **18**: 165. 1916.

C. panamense var. *macrophyllum* Standley, Field Mus. Pub. Bot. **22**: 366. 1940.

Tree about 6–15 m. high; leaves finely white-strigulose beneath when young, soon glabrate, elliptic or elliptic-obovate, broadest near or above the middle, commonly but not always cuneate or acutish at the base (less so when larger) and abruptly acuminate at the apex, of various sizes up to about 30 cm. long and 14 cm. wide, usually much smaller, but averaging larger than in other North American species; flowers numerous in axillary clusters, the pedicels about 3–7 mm. long, closely cinereous; calyx about 1.0–1.5 mm. long, but appearing longer from the outside because of the thickened receptacle, closely strigulose with grayish or slightly rufous hairs, the two outer sepals tending to be obtuse or acutish, the others broadly rounded; corolla pale green to yellowish or nearly white, about 4.1–5.2 mm. long, with a sparsely or moderately short-hairy patch near the base of each lobe and extending to the top of the tube, or the patches occasionally confluent on the tube; free part of the corolla-lobes only about 1.1–1.5 mm. long, about 1/4–2/5 as long

as the tube, but the creases showing clearly on the outside for about as long as the free part; anthers about 0.4–0.6 mm. long; filament-traces, or some of them, evident; stigma distinctly 5-lobed; fruit purple or reddish-purple, generally several-seeded, from oblate-spheroidal (pumpkin-shaped) to very broadly ellipsoid or ovate and pointed at the end, about 1–2.5 cm. long and 1.2–2.0 cm. thick; seed-scar basilateral, extending to the middle of the seed or beyond.

TYPE: *Pittier 4005*, Sirri River, Trinidad basin, Colon, Panama Canal Zone, near sea level, July 20, 1911 (G, US).

DISTRIBUTION: Panama Canal Zone to Costa Rica, chiefly on the Atlantic slope.

COSTA RICA: *Stork 1669* (F, Y). Cartago: *Tonduz 12852* (= Donn. Smith dist. #7572) (US). PANAMA: Bocas del Toro: *Cooper 353* (F, NY, Y); *Hart 150* (US). Coele: *Williams 276* (NY, US). CANAL ZONE: Gatun: *Hayes s.n.* (Feb. 1, 1860) (A, F, G, MO, NY, US). Barro Colorado Island: *Aviles 969* (F); *Bailey & Bailey 397* (F); *Bangham 591* (A, F, US); *Salvoza 981* (A); *Shattuck 1024* (F); *Starry 82* (F); *Starry 118* (F); *Wetmore & Abbe 169* (A, F, G); *Zetek 3810* (F, MO); *Zetek 4327* (F); *Zetek 4328* (F); *Zetek 4330* (A, NY, F); *Kenoyer 494* (US).

The variety *macrophyllum* seems to me to consist merely of the larger-leaved specimens in herbaria, rather than representing a real entity.

In Costa Rica this species might be confused with *C. brenesii*, which has a narrower apparently single-seeded fruit, smaller and narrower leaves, and smaller flowers with obscure filament-traces, and lacks the thin layer of tissue that joins the lower half of the corolla-lobes in *C. panamense*.

5. CHRYSOPHYLLUM BICOLOR Poir. Encyc. Suppl. **2**: 15. 1811.

C. eggersii Pierre, Symb. Ant. **5**: 155. 1904.

Small tree; leaves conspicuously reddish-sericeous beneath when young, later glabrate, the hairs sometimes fading before falling; leaves elliptic-ovate or elliptic, broadest near or below the middle, rounded to acutish at the base, acute to acuminate at the tip, up to about 13 cm. long and nearly 6 cm. wide; flowers several in each cluster, the pedicels 3–10 mm. long and closely or somewhat loosely rufous-hairy; calyx about 1.0–1.7 mm. long, the sepals broad, obtuse or rounded, closely rufous-hairy outside, and slightly so within, some of the inner ones commonly with smooth somewhat petaloid narrow margins; corolla thick and firm, about 3.5–4.7 mm. long, generally appearing relatively broader than in related species, the lobes (except for the margins) and upper part of the tube conspicuously rufous-hairy, more generally and evenly so than in *C. pauciflorum;* corolla-lobes shorter than the tube, at least as measured from the inside, sometimes equaling or slightly exceeding it as measured from the outside; filament-traces obscure; anthers about 0.6–0.8 mm. long; stigma about 5–9-lobed, the ovary with as many locules; fruit ovoid or subglobose, or reported to be sometimes pyriform, about 2–3 cm. long and 1.5–2 cm. thick, with one or occasionally several seeds; seeds relatively short and thick, the scar basilateral, extending to the middle or beyond.

TYPE: *Ledru*, Puerto Rico.

DISTRIBUTION: Puerto Rico and the Virgin Islands.

PUERTO RICO: *Stevenson 2151* (US). ST. THOMAS: *Britton & Britton 220* (NY); *Eggers s.n.* (July 18, 1876) (NY, G, MO); *Eggers s.n.* (July 21, 1876) (NY); *Eggers s.n.* (July 29, 1876) (G, MO). ST. CROIX: *Rickseeker 369* (G, MO, NY, US).

6. CHRYSOPHYLLUM CAINITO L. Sp. Pl. 192. 1753.

C. cainito var. *pomiferum* Pierre, Symb. Ant. 5: 154. 1904.

Small to medium-sized or large tree sometimes as much as 30 m. high; leaves soon glabrous, shining, and usually with evident slightly raised veins above, densely rufous-sericeous or -tomentulose beneath, sometimes becoming a little paler in age, elliptic or oblong, obtuse or acutish at the base, generally abruptly short-acuminate at the apex, up to about 16 cm. long and 8 cm. wide; flowers numerous in axillary clusters, the pedicels closely rufous-hairy, generally about 1 cm. long or a little more; calyx about 1.0–1.2 mm. long, closely rufescent, the lobes suborbicular; corolla greenish or yellowish, about 3–5 mm. long, the tube glabrous, the lobes rufous-hairy except near the margins, somewhat ovate, more tapering than in most species, equaling or usually a little exceeding the tube; anthers about 0.7–0.9 mm. long; filaments attached on the corolla-lobes near the base, their traces obscure; stigma discoid, about 7–12-lobed, the style very short, fruit yellow, green, or purple, large, 3 cm. or more thick, reported to reach or exceed 10 cm. thick, commonly subglobose, several-seeded; seeds flattened, obliquely obovate, with a broad essentially lateral scar extending nearly the length of the seed.

TYPE: Linnaeus cites no specimens, but refers to several other publications.

LOCAL NAMES: Star-apple, caimito.

DISTRIBUTION: Probably indigenous throughout the West Indies, where also cultivated; cultivated and naturalized throughout Central America and southern Mexico.

Citation of specimens seems superfluous.

7. CHRYSOPHYLLUM TEPICENSE Standley, Contr. U. S. Nat. Herb. **23**: 1115. 1924.

"Branchlets brownish-tomentulose; leaves (very immature) on petioles 1.5 to 2 cm. long, the blades oblong-elliptic, 3.5–4 cm. long, 1.7–2 cm. wide, obtuse or rounded at base and apex, densely brown-tomentulose on both surfaces; flowers borne on defoliate nodes of old branchlets, numerous in each cluster, the pedicels 8 to 10 mm. long, puberulent; sepals 5, orbicular, 2 mm. long, minutely sericeous; corolla 3.5 mm. long, greenish, sparsely sericeous; fruit oval, about 3 cm. long and 2 cm. thick, 1-seeded; seed brown, smooth, about 2.2 cm. long, the hilum near the apex on the ventral side, 8 mm. long and 3.5 mm. wide." Description copied from the original; no specimens seen.

TYPE: *Rose 1456*, Acaponeta, Tepic, Mexico.

DISTRIBUTION: Known only from the type collection.

8. **Chrysophyllum hirsutum** Cronquist, sp. nov.

Shrub or small tree 3–7 m. high; leaves loosely rufous-hirsutulous on both sides when young, soon becoming glabrous, dark green, and shining above, more permanently hairy beneath, though perhaps eventually glabrate there too in old leaves, the hairs beneath a little shorter and stiffer than those above, sometimes fading a little in age; twigs of the season's growth loosely

rufous-hirsutulous; leaves elliptic, rounded to usually acuminate (sometimes abruptly so) at the apex, rounded to acute at the base, up to about 11 cm. long and 5.5 cm. wide; petioles less than 1 cm. long; flowers about 2–6 in each axillary cluster, the pedicels densely rufous-hairy, about 4–8 mm. long; calyx about 2.0–3.0 mm. long, densely and loosely rufescent, the lobes narrowly to broadly triangular-ovate, the outer ones generally hairy within near the margins and broader than the inner; corolla about 4.5–5.5 mm. long, rufous-hirsute with appressed hairs above the middle or to near the base; corolla-lobes nearly or quite half as long as the tube, as measured from the inside, a little longer outside; anthers about 0.8–1.0 mm. long; filament-traces obscure or sometimes visible; filaments connected by a slightly thickened ring in the corolla-throat; stigma 5-lobed, or, in 4-lobed corollas, 4-lobed; ovules attached laterally below the middle, in flower; immature fruit about 2.6 cm. long and 1.0 cm. thick, broadest about 1 cm. from the tip, apparently single-seeded.

Frutex vel arbor parva, petiolis minus quam 1 cm. longis, foliis ellipticis plerumque acuminatis usque ad 11 cm. longis 5.5 cm. latis utrinque hirsutulis pilis rufis laxis patentibus, supra mox glabratis nitidis, infra pilis persistentibus vel tarde deciduis, pedicellis ca. 2–6, 4–8 mm. longis, calyce ca. 2.0–3.0 mm. longo dense et laxe hirsutulo pilis rufis, corolla ca. 4.5–5.5 mm. longa extra pubescente pilis appressis rufis, lobis dimidio tubo aequalibus, antheris ca. 0.8–1.0 mm. longis, stigmate 5-lobato, drupis (immaturis) anguste obovoideis 2.6 cm. longis 10 mm. crassis.

TYPE: *Austin Smith 1776*, Villa Quesada, San Carlos, Alajuela, Costa Rica, 825 m., Caribbean rain forest zone, at edge of forest, March 14, 1939 (A); ISOTYPE (F).

DISTRIBUTION: Alajuela, Costa Rica.

COSTA RICA: Alajuela: *Austin Smith 1922* (A, F, MO); *Austin Smith P2593* (Kr, NY).

9. CHRYSOPHYLLUM CLARAENSE Urb. Rep. Sp. Nov. **21**: 68. 1925.

"Frutex v. arbor parva. Rami terets cinerei, internodiis 0.5–1.5 mm. longis. Folia 4–6 mm. longe petiolata, obovata vel obovata-elliptica, inferne subsensim angustata, apice obtusiuscula v. obtusa, 2.5–4 cm. longa, 1–2 cm. lata, nervo medio per totam longitudinem impresso, lateralibus crebris supra vix conspicuis, subtus sub tomento absconditis, supra nitida ab initio glabra in sicco olivacea, subtus tomento ferrugineo posterius pallescente obtecta, coriacea. Flores non visi. Fructus (unicus tantum obvius) 5 mm. longe pedicellatus, globulosus perpaullo longior quam crassus, 8 mm. longus, 7.5 mm. crassus." Description copied from the original; no specimens seen.

TYPE: *Ekman 18891*, "in Cuba prov. Santa Clara prope Casilda in fruticetis litoralibus solo araneose, m. Mart. fruct."

DISTRIBUTION: Known only from the type collection.

10. CHRYSOPHYLLUM OLIVIFORME L. Syst. ed. 10. **2**: 937. 1759.

Shrub or more commonly a small tree, sometimes as much as 20 m. high; leaves much like those of *C. cainito*, glabrous and shining above, densely rufous-sericeous or tomentulose beneath, or occasionally relatively pale beneath, elliptic or oblong to nearly ovate, obtuse to acute or more commonly

abruptly short-acuminate at the apex, averaging a little smaller than in *C. cainito*, seldom more than about 10 cm. long and 5 cm. wide (rarely to 14 cm. × 8 cm.), and with the veins less evident and scarcely raised on the upper surface; flowers numerous to few or even solitary in the axils, the pedicels closely rufous-hairy, mostly about 4–7 mm. long; corolla yellowish or greenish, about 3.1–5.0 mm. long, glabrous or occasionally the upper part of the tube rufous-hairy, the lobes distinctly shorter than the tube, as measured from the inside; filaments inserted at about the level of the sinuses, connected by a slightly thickened ring in the corolla-throat, the traces obscure or occasionally evident; anthers commonly 0.6–0.8 mm. long; stigma distinctly 5-lobed; fruit reported to be purple, ellipsoid, single-seeded, about 1–2 cm. long and up to half as thick, distinctly oblique toward the base, at least in herbarium specimens, because of the broad basilateral seed-scar, which does not extend to the middle of the fruit.

TYPE: None given.

DISTRIBUTION: Common in thickets and scrub forest; southern Florida, the Bahama Islands, and the Greater Antilles.

LOCAL NAMES: Satin leaf, teta de burra, caimitillo, saffron tree, caimitier maron.

1. Leaves usually over 5 cm. long, often but not always at least half as wide as long; flowers generally several or numerous in a cluster ..... *C. oliviforme* var. *typicum*.

1. Leaves generally 5 cm. long or less, and less than half as long as wide, or if sometimes more than 5 cm. long, then not more than $\frac{2}{3}$ as long as wide; flowers about 1–6 in a cluster ..... *C. oliviforme* var. *picardae*.

10A. CHRYSOPHYLLUM OLIVIFORME var. typicum Cronquist, var. nov.

C. oliviforme L. Syst. ed. 10. **2**: 937. 1759, sens. strict.
C. oliviforme var. *pallescens* Urb. Symb. Ant. **5**: 157. 1904.
C. oliviforme var. *platyphyllum* Urb. l. c.
C. brachycalyx Urb. Symb. Ant. **7**: 327. 1912.
C. pallescens Urb. Rep. Sp. Nov. **13**: 470. 1915.
C. platyphyllum Urb. Rep. Sp. Nov. **13**: 470. 1915.
C. gonavense Urb. Ark. Bot. **22A**(17): 74. 1929.
C. miragoanеum Urb. Arkiv. Bot. **22A**(17): 75. 1929.

Characters as in the key. Range of the species.

UNITED STATES: Florida: *Bessey 81* (A); *Bessey 105* (A, MO); *Bessey 114* (A, MO); *Britton 383* (NY); *Chapman s.n.* (1875) (G, MO, US); *Cowles 909* (US); *Curtiss 1758* (A, G, MO, NY, US); *Duckett 201* (A, NY, US); *Eyles 8156* (G); *Fredholm 5557* (G); *Garber s.n.* (US, G, MO); *J. A. Harris C21272* (US); *A. H. Howell 865* (US); *A. H. Howell 1021* (US); *Killip 32875* (A); *McFarlin 3836* (Mich); *McFarlin 9728* (NY); *Moldenke 5604* (NY); *Muenscher & Muenscher 14120* (NY); *Muenscher & Muenscher 14182* (A); *Rehder 890* (A); *Rhoads 8314* (A); *Safford & Mosier 40* (US); *Seibert 1171* (A, MO); *Simpson 387* (G, NY); *Small 2117* (NY); *Small 2183* (NY); *Small 7673* (NY); *Small 8760* (NY); *Small & Carter 1236* (NY); *Small & Carter 2648* (NY); *Small & Mosier 5903* (NY); *Small & Mosier 6026* (G, MO, NY); *Small, Mosier & Dewinkeler 10964* (US); *Small, Mosier & Thompson 5779* (NY); *Tidestrom 4222* (MO); *Webber 66* (MO). BAHAMAS: *Brace 239* (F); *Brace 6856* (NY); *Britton 134* (NY); *Britton 3266* (NY, MO, US); *Curtiss 31* (A, G, MO, NY, US); *Eggers 4468* (US); *Northrop & Northrop 262* (A, F, G, NY); *Wilson 7724* (NY, MO, G). CUBA: *Gill & Whitford 41* (Y); *Gill & Whitford 84* (Y); *Griffith 37* (NY); *Griffith 64* (NY); *Rehder 1201* (A); *Wright 344* (MO, G); *Wright 1322* (G, NY, MO, US). Pinar del Rio: *Earle*

& Wilson 1631 (NY); *Earle & Wilson 1633* (NY); *Earle & Wilson 1634* (NY); *Shafer 10470* (NY, MO, US); *Van Hermann 855* (US); *Wilson 1210* (NY, US); *Wilson 9367* (NY); *Wilson 11575* (NY, MO, US). Isla de Pinos: *Palmer & Riley 1021* (NY, US); *Rowlee 218* (NY). Habana: *Baker & Wilson 282* (NY); *Earle & Wilson 2395* (NY); *Shafer 17* (NY); *van Hermann 155* (NY); *van Hermann 309* (MO); *van Hermann 397* (NY, MO, US); *van Hermann 468* (A, NY, MO, US); *van Hermann 5059* (A, G, US); *Wilson 1001* (NY, US); *Wilson 1002* (NY); *Wilson 1071* (NY, US); *Wilson 1989* (NY); *Wilson 3527* (NY); *Wilson 9464* (G, NY). Matanzas: *Britton, Britton & Shafer 547* (NY); *Britton & Wilson 26* (NY); *Britton & Wilson 253* (NY); *Rugel 5a* (NY); *Rugel 774* (NY). Santa Clara: *Combs 234* (F, G, MO, NY); *Combs 361* (F, G, MO, NY); *Hodge, Howard & Godfrey 4166* (G, MO, NY, US); *Hunnewell 11503* (G); *Jack 4257* (A); *Jack 4629* (A, MO, US); *Jack 5482* (A, US); *Jack 5895* (A, US); *Jack 7480* (A, US); *Jack 8138* (A, US); *Salvoza 561* (A); *L. B. Smith & Hodgdon 3206* (F, G, Mich, MO, NY, US). Camaguey: *Shafer 57* (NY, US); *Shafer 2903* (NY, US). Oriente: *Matthews & Crosby 30* (Y); *Ekman 10228* (NY). JAMAICA: *Fredholm 3290* (NY); *Harris 5363* (A, NY, US); *Harris 5562* (G); *Harris 5909* (A); *Harris 9955* (US); *March 795* (G); *March 1467* (NY). HAITI: *Barbour 32* (Y); *Ekman H1819* (US); *Ekman H2277* (US); *Ekman H2336* (US); *Ekman H2388* (US); *Ekman H5113* (US); *Ekman H6512* (US); *Ekman H8398* (US); *Ekman 8648* (US); *Eyerdam 237* (G, US); *Eyerdam 295* (A, G, MO, NY, US); *Eyerdam 331* (G, MO, US); *Holdridge 1341* (US); *Leonard 3357* (G, NY, US); *Leonard 5163* (US); *Leonard 7031* (G, NY, US); *Leonard 7710* (G, NY, US); *Leonard 8449* (NY, US); *Leonard & Leonard 11148* (Mich, US); *Leonard & Léonard 11273* (MO, US); *Leonard & Leonard 11475* (A, US); *Leonard & Leonard 11643* (G, NY, US); *Leonard & Leonard 12663* (A, G, US); *Leonard & Leonard 12735* (US); *Leonard & Leonard 12935* (US); *Leonard & Leonard 13573* (NY, MO, US); *Leonard & Leonard 14859* (US); *Miller 311* (US); *Nash 727* (NY); *Nash & Taylor 1308* (NY, US). DOMINICAN REPUBLIC: *Jaeger 185* (US); *Eggers 2446* (A, NY, US); *Ekman 13113* (US); *Ekman 15767* (US); *Fuertes 65* (NY); *Fuertes 858* (A, G, US); *Fuertes 1057* (A, MO); *Fuertes 1311* (A); *Fuertes 3011* (G, MO, NY, US); *Rose, Fitch & Russell 3794* (NY, US); *Rose, Fitch & Russell 4182* (G, NY, US); *Rose, Fitch & Russell 4349* (US); *Scarff 11* (F); *Taylor 168* (NY); *Taylor 420* (NY); *Turckheim 3608* (G, MO, NY, US); *Wright, Parry & Brummel 201* (US); *Wright, Parry & Brummel 203* (G). PUERTO RICO: *Britton & Britton 7887* (NY); *Britton & Britton 8005* (G, NY, US); *Britton & Britton 8030* (G, NY, US); *Gregory 344* (PR); *Heller 4426* (A, G, MO, NY, US); *Miller 1645* (US); *Sargent 302* (US); *Sargent 507* (US); *Sintenis 685* (G, US); *Sintenis 2641* (US); *Underwood & Griggs* 162 (G).

10B. CHRYSOPHYLLUM OLIVIFORME var. **picardae** (Urb.) Cronquist, comb. nov.

C. picardae Urb. Symb. Ant. **5**: 158. 1904.
C. brachystylum Urb. Symb. Ant. **7**: 327. 1912.
C. heterochroum Urb. Ark. Bot. **22A**(17): 74. 1929.

Characters as in the key; leaves sometimes becoming narrowly obovate, with tapering cuneate base.

TYPE: *Picarda 1198*, "Hab. in Haiti prope Port-au-Prince, m. Mart. fruct."

DISTRIBUTION: Haiti and the Dominican Republic; apparently not common.

HAITI: *Ekman H2183* (US); *Ekman 6748* (US); *Ekman H5077* (US).

The var. *picardae* is well marked by its small relatively narrow leaves and few flowers, but passes readily into the more usual forms of the species. It might be regarded as transitional between *C. oliviforme* and *C. angustifolium*, but the rather scanty material available does not indicate any intergradation with the latter.

It has been remarked by collectors that in Florida the phase of the species that grows in pine woods seems different from the hammock phase, having the pubescence of the lower side of the leaves much lighter-colored. No clear-cut segregation of the species as a whole on this basis seems feasible, at least in the herbarium.

11. CHRYSOPHYLLUM MEXICANUM Brand. ex Standley, Contr. U. S. Nat. Herb. **23**: 1114. 1924.

Large shrub or more commonly a small to medium-sized tree, mostly 2–15 m. high, occasionally as much as 25 m.; leaves elliptic or elliptic-oblong to sometimes elliptic-ovate, acutish or abruptly short-acuminate at the apex, up to about 14 cm. long and 5 cm. wide, glabrous and shining above, the lateral veins sometimes prominent and a little raised, sometimes not, closely and relatively finely sericeous-strigose beneath, the lateral veins showing clearly through the pubescence, the hairs varying from more or less rufous to pale, the lower leaf-surface averaging considerably paler than in *C. cainito* or *C. oliviforme*, but usually redder than in *C. argenteum*, sometimes nearly glabrate in age, or occasionally soon glabrate, the hairs then very fine and inconspicuous; flowers numerous in clusters axillary to a leaf or leaf scar, in the latter case inferior to a leafy and floriferous branch of the season; pedicels closely hairy to occasionally subglabrous, mostly 3–5 mm. long; flowers averaging distinctly smaller than in *C. oliviforme;* calyx about 0.8–1.6 mm. long; corolla reported to be greenish to white, about 2.2–3.5 mm. long, the lobes equaling or generally a little exceeding the tube, as measured from the inside, commonly slightly auriculate; filaments commonly inserted on the corolla-lobes a little above the level of the sinuses, the thickened ring absent or obscure; anthers mostly 0.4–0.6 mm. long, stigma distinctly 5-lobed; fruit reported to be yellow to brown or black, ellipsoid, single-seeded, about 1–2 cm. long and half as thick, oblique at the base, at least in herbarium specimens, because of the broad basilateral seed-scar.

TYPE: *Purpus 7679*, Zacuapan, Vera Cruz, Mexico, 900–1000 m., Sept. 1916 (A, MO, US).

DISTRIBUTION: Tamaulipas and San Luis Potosi, Mexico, to Honduras and El Salvador.

LOCAL NAMES: caimito, zapote caimito, canela, palo de canela, piste, chike, siciya, zayoyillo, guayabillo, guava de danto, damsel.

1. Leaves early glabrate, the hairs fine and relatively inconspicuous, not rufescent; pedicels sparsely strigose or subglabrous; Oaxaca ..... *C. mexicanum* var. *politum*.

1. Leaves tardily or not at all glabrate, the hairs coarser and forming an evident silky covering, often more or less rufescent; pedicels more evidently hairy; general from Tamaulipas and San Luis Potosi, Mexico, to Honduras and El Salvador ..... *C. mexicanum* var. *typicum*.

11A. CHRYSOPHYLLUM MEXICANUM var. **typicum** Cronquist, var. nov.

C. mexicanum Brand. ex Standley, Contr. U. S. Nat. Herb. **23**: 1114. 1924, sens. strict.

Characters as in the key. Range of the species.

MEXICO: *Karwinski 4243* (NY—Photo); *Sessé, Mociño, Castillo & Maldonado 5104* (F). Tamaulipas: *Palmer 321* (NY, US). San Luis Potosi: *Edwards 645* (F, MO). Vera Cruz: *Purpus 8038* (G, MO, NY, US); *Purpus 8258* (A, G, MO, NY, US); *Purpus 8258a*

(G, MO, NY, US); *Purpus 10775* (A, MO, F, Mich, US); *Williams 8643a* (F). Oaxaca: *Liebmann 308* (F, NY, US); *Liebmann 12343* (F); *Reko 3082* (US); *Reko 3498* (US). Yucatan: *Gaumer 896* (A, F, G, MO, NY, US); *Gaumer 24272* (A, F, G, NY, MO); *Lundell 1190* (A, F, G, Mich, MO, NY, US); *Lundell 1246* (F, G, Mich, MO, NY, US); *Lundell & Lundell 7561* (A); *Millspaugh 1555* (F); *Steggerda 40a* (F); *Stewart 257* (G). Quintana Roo: *Lundell & Lundell 7639* (A). Chiapas: *Matuda 2621* (A, F, Mich, NY); *Matuda 4768* (A, MO, NY); *Purpus 167* (US); *Purpus 10167* (NY, US). GUATEMALA: El Peten: *Bartlett 12276* (Mich, US); *Lundell 3501* (Mich); *Mercedes 396* (Mich, MO). Alta Verapaz: *Standley 70149* (A, F). Izabal: *Standley 23942* (US). Quezaltenango: *Skutch 1333* (A, NY, F, US). BRITISH HONDURAS: *Castillo 21* (F, Y); *Gentle 10* (F); *Gentle 149* (A, F, Mich, NY, US); *Gentle 245* (F, Mich, US); *Gentle 1821* (A, F, Mich, MO, NY); *Gentle 2975* (A, F, Mich, NY, US); *Gentle 3085* (NY, A, Mich); *Gentle 4092* (A, MO); *Gentle 4744* (NY); *Heyder 32* (US, Y); *Karling 53* (NY, A, US); *Kluge 20* (Y, US); *Lundell X11* (US); *Lundell LP12* (F); *Lundell 4744* (Mich, MO); *Lundell 6415* (NY, Mich); *Meyer 83* (F, Mich); *Schipp 303* (A, F, G, Mich, MO, NY, US); *Schipp 753* (A, F, G, Mich, MO, NY); *Winzerling V–2* (F, US, Y). HONDURAS: Camayagua: *Standley 56505* (A); *Yuncker, Dawson & Youse 5730* (F, G, Mich, MO, NY). Tegucigalpa: *von Hagen & von Hagen 1145* (NY). Yoro: *Edwards 632* (A, F, US); *Standley 55060* (A, US). Atlantida: *Standley 54747* (A, US); *Standley 54751* (A, US); *Standley 55179* (A, US); *Standley 56868* (A, US). EL SALVADOR: *Calderon 1088* (G, NY, US); *Calderon 1393* (US). Ahuachapan: *Standley 20048* (US).

11B. CHRYSOPHYLLUM MEXICANUM var. **politum** Cronquist, var. nov.

A var. typico differt foliis infra mox glabratis, pedicellis subglabris vel sparse strigosis.

TYPE: *Martinez-Calderon 241*, shrub in llanos, Chiltepec and vicinity, district of Tuxtepec, Oaxaca, Mexico, about 20 m.; July, 1940–February, 1941 (A).

DISTRIBUTION: Known only from the type collection, Oaxaca, Mexico.

The difference in pubescence between var. *politum* and the more usual forms of *C. mexicanum* is very pronounced, but seems not to be correlated with any other differences, so that it seems unlikely that the character is of more than varietal importance.

This species has sometimes been confused with its West Indian relative, *C. oliviforme*, from which it differs in its smaller flowers of somewhat different proportions and in the generally finer and lighter-colored pubescence of the lower side of the leaves, as well as several minor features such as the more abundant flower-clusters, often more numerous flowers, and the frequent occurrence of the flower clusters beneath lateral shoots. I have seen no evidence of intergradation, nor is there any range-overlap, and the two scarcely seem conspecific.

12. CHRYSOPHYLLUM ANGUSTIFOLIUM Lam. Tab. Encyc. **2**: 44. 1793.

C. montanum Urb. Rep. Sp. Nov. **3**: 469. 1915.

Tree; leaves narrowly elliptic or elliptic-lanceolate, acuminate at both ends, mostly about 4–7 cm. long and 10–20 or 25 mm. wide, commonly 3–5 times as long as wide, the larger ones sometimes a little wider, green and shining above, but commonly with some inconspicuous cobwebby-villous white or slightly reddish hairs, at least until maturity, when often glabrate,

densely villous-tomentose with rufous hairs beneath, the veins more or less evident on the upper surface, and generally pointing more nearly forward than in other species, but highly variable in that respect, chiefly according to the relative width of the leaf; flowers few or solitary in axillary clusters, the strongly rufous hairy pedicels mostly 5–8 mm. long; calyx densely rufous-hairy, about 1.5–1.8 mm. long, the outer sepals tending to be deltoid-ovate and acute or acutish, the inner ones broad, rounded, and shorter; corolla about 4.4–4.9 mm. long, the lobes shorter than the tube, which generally has a few hairs on the outside near the sinuses; anthers about 0.5–0.8 mm. long, the thickened ring more or less evident or sometimes obscure; filament-traces obscure; stigma 5-lobed; fruit single-seeded, ellipsoid, similar in size and shape to that of *C. oliviforme*.

TYPE: None given, but stated to come from Santo Domingo. Possibly the Martin collection cited by Pierre and Urban.

DOMINICAN REPUBLIC: *Fuertes 630* (NY, US); *Fuertes 1296* (A, G, US).

DISTRIBUTION: Haiti and the Dominican Republic.

This apparently rare species is related to *C. oliviforme*.

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DESCRIPTIONS OF TROPICAL RUSTS—VII[1]

GEORGE B. CUMMINS

The Uredinales reported in this paper were collected, for the most part, by C. G. Hansford in Uganda and by F. C. Deighton in Gold Coast and Sierra Leone and were made available through the courtesy of Miss E. M. Wakefield and Dr. G. R. Bisby. Of the species from other regions, four were collected in Australia by Mrs. Clemens and one was intercepted in customs as from Venezuela. Type specimens are deposited in the Arthur Herbarium of the Purdue University Agricultural Experiment Station. African specimens are also in the herbaria of either the Royal Botanic Gardens or the Imperial Mycological Institute, Kew and the Australian collections are in the Queensland Herbarium, Botanic Gardens, Brisbane.

PHAKOPSORA HANSFORDII Cumm. On *Alcornea cordifolia*, UGANDA: Kawanda, June 1939, *Hansford 2474;* on *Acalypha* sp., UGANDA: Kigezi, Aug. 1937, *Hansford 2152.*

Pycnia, aecia, uredia, and telia are present in No. 2474. The telia are so closely associated with the aecial infections as to strongly indicate that all sori belong in the life cycle of the species. The aecial stage is as follows:

Pycnia amphigenous, or mainly epiphyllous, subcuticular, lenticular or usually applanate, 30–35 μ high, 65–110 μ wide, hymenium flat, aparaphysate. Aecia amphigenous, occurring along the veins with accompanying hypertrophy involving lengths up to 1.5 cm. or in small scattered groups. Peridium nearly colorless, 165–190 μ in diameter and up to 1.0 mm. in length but fragile, lacerate, and usually broken at the host; peridial cells cuboid, rhomboid or oblong, 15–19 × 20–30 μ, inner wall moderately verrucose, 2.5–3 μ thick, outer wall punctate-striate, 2.5–3 μ thick, cells abutted or slightly overlapping, aeciospores ellipsoid or oblong-ellipsoid, 15–18 × 19–25 μ; wall pale yellowish brown or nearly hyaline, 1–1.5 μ thick, finely verrucose.

If the aecia actually belong in the life cycle, *P. hansfordii* is the first species of *Phakopsora* known to have cupulate aecia. Mundkur (Mycologia **35**: 542. 1943) has reported uredinoid aecia, unaccompanied by pycnia, as occurring in *P. stereospermi* Mundkur. He describes the aecia as lacking peridia and paraphyses and as having catenulate, echinulate aeciospores. Examination of a portion of the type specimen, furnished by Dr. Mundkur, leads me to question his description. The sori appear to be ordinary uredia (*Uredo stereospermi* Syd., in fact) with pedicellate spores produced on well-developed, brown, sporogenous basal cells. The telia are crustose with the spores strictly catenulate. *P. stereospermi* is not a species of *Phakopsora.*

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PHAKOPSORA APODA (Har. and Pat.) Mains. On *Pennisetum polystachyum*, UGANDA, Pallisa, Bugwere, Jan. 1930, *Hansford 1024*; on *Setaria aequalis*, UGANDA: Kampala, Feb. 1930, *Hansford 1059*.

Uredia and telia are present on the *Pennisetum* but only uredia on *Setaria*. The characteristics of the uredia indicate that the rust on these hosts is identical with or closely related to *P. apoda*. Mains (Mycologia **30**: 45. 1938) has published photographs of telia from the original collection made in French Congo. No other reports of the species have been published.

Phakopsora grewiae (Pat. and Har.) Cummins, comb. nov. (fig. 1) (*Uredo grewiae* Pat. and Har. Jour. de Bot. **14**: 237. 1900). On *Grewia* sp., Thies (Cayor), Senegal, *Chevalier*.

Telia amphigenous, scattered or in small groups, blackish brown, subepidermal in origin but becoming erumpent, pulvinate or short cylindric, round, 64–165 μ in diam., 100–140 μ high; teliospores without definite arrangement, varying in size and shape, the marginal spores usually cuboid or globoid, the central cells usually oblong, 8–14 × 11–23 μ; wall cinnamon- or chestnut-brown, darkest in the outer spores, 1.5–2 μ thick, the apex uniform or thickened to 3 μ, smooth.

This specimen, a part of the Patouillard herbarium and made available through the courtesy of Dr. D. H. Linder, was collected in the type locality (Thies). The species is a typical *Phakopsora* and differs from *Uredo corbiculoides*, described below, for reasons enumerated under that species.

Phakopsora desmium (Berk. & Br.) Cummins, comb. nov. (*Aecidium desmium* Berk. & Br. Jour. Linn. Soc. **14**: 95. 1875; *Uredo gossypii* Lagerh. Jour. Mye. **7**: 48. 1891; *Kuehneola gossypii* Arth. N. Am. Flora **7**: 187. 1912; *Uredo desmium* Petch Ann. Bot. Gard. Peradeniya **5**: 247. 1912; *Cerotelium gossypii* Arth. Bull. Torrey Club **44**: 510. 1917; *Kuehneola desmium* Butl. Fungi & Dis. Plants 363. 1918).

An examination of the specimen (on *Gossypium acuminatum*, Santiago, Cuba, Mar. 1903, *L. M. Underwood & F. S. Earle 159*) upon which Arthur (*l.c.*) based his description of telia and his transfer to the genus *Kuehneola* proves that the teliospores are not catenulate but lack special orientation. The telia are typically phakopsoroid.

Angiopsora hansfordii Cummins, sp. nov. Pycniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, sparsis vel laxe aggregatis, ovalis vel oblongis, 0.3–1.0 mm. longis, flavidis; paraphysibus peripherales incurvatis, hyalinis vel pallide flavidis, 9–15 × 25–40 μ; membrana 1–1.5 μ cr., ad apicem 2–5 μ cr.; urediosporae late ellipsoideae, ellipsoideae vel obovoideae, 14–19 × 20–27 μ; membrana pallide flavida, 1.5–2 μ cr., minuteque verrucoso-echinulata; poris germ. obscuris. Teliis inter urediis sparsis, castaneis, subepidermalibus, indehiscentibus, ovalis vel oblongis, 0.3–1.0 mm. longis; teliosporis plerumque 2 superpositis, cuboideis vel oblongis, 9–17 × 18–30 μ; membrana pallide castaneo-brunnea, 1.5 μ cr., ad apicem 2–5 μ cr., levi.

On *Melinis tenuissima*, UGANDA: Kyasoweri, Elgon, Dec. 1933, *Hansford 1714* (TYPE).

Uredo melinidis Kern, the only other rust described on the genus *Melinis*, has cinnamon-brown urediospores with equatorial pores and sori without paraphyses. No species of *Angiospora* has been reported from Africa previously.

CROSSOPSORA FICI Arth. & Cumm. On *Ficus capensis*, UGANDA: Mukono, Kiagwe, June 1942, *Hansford 3051*.

This species, previously known only from the type collected in Luzon on *Ficus variegatus*, is characteristic because of the cinnamon-brown, angularly obovoid urediospores having three pores located in the angles. Telia are not present in Hansford's specimen.

CROSSOPSORA ANTIDESMAE-DIOICAE (Racib.) Arth. and Cumm. On *Antidesma venosa*, UGANDA: Entebbe Road, June 1942, *Hansford 3012*.

The species has not been reported from Africa previously.

Cerotelium deightonii Cummins, sp. nov. Urediis hypophyllis, subepidermalibus, sparsis, flavidis, rotundatis, 165–250 μ diam.; peridio hyalino fragili hemisphaerico dein poro aperto cinctis; urediosporae oblongae, obovoideae vel late ellipsoideae, 13–18 × 20–30 μ; membrana 1–1.5 μ cr., ad apicem usque ad 5 μ cr., aureo-brunnea, moderate echinulata; poris germ. 2, aequatorialibus. Teliis urediis similibus sed eperidiatis; teliosporis 6–10 catenatim superpositis, plerumque oblongis, 11–15 × 13–24 μ; membrana hyalina, 0.5–1 μ cr., levi; statim germinantis ad apicem in basidium typicum; basidiosporae globoideae, 7–9 μ diam.

On *Phyllanthus discoideus*, SIERRA LEONE: Makene, January 27, 1939, *Deighton 1772* (TYPE).

Pycnia and aecia, apparently *Aecidium phyllanthi* P. Henn., in sufficiently intimate association to suggest relationship occur on the leaves with the uredia and telia. The subcuticular pycnia and cupulate aecia correspond in structure to those of *C. dicentrae* (Trel.) Mains and Anders. but lack the systemic habit of that species.

Pycnia abundant, subcuticular, hypophyllous, lenticular to applanate, 120–165 μ diam., hymenium flat, aparaphysate. Aecia on brown spots, in groups reaching a diameter of 1.3 mm., cupulate, yellowish, the margin recurved, 0.3–0.5 mm. diam., aeciospores globoid or broadly ellipsoid, 16–20 × 20–25 μ; wall 1–1.5 μ thick, hyaline or nearly so, verruculose.

UROMYCES LEPTODERMUS Syd. On *Brachiaria platynota*, UGANDA: Kawanda, July 1940, *Hansford 2766;* on *Panicum deustum*, UGANDA: Kawanda, Aug. 1940, *Hansford 2772;* on *Setaria pallide-fusca*, UGANDA: Kisoro and Kabale, Kigezi, Aug. 1937, *Hansford 2192, 2228;* on *Setaria rubiginosa*, UGANDA: Kampala, Jan. 1930, *Hansford 1051;* on *Setaria verticillata*, UGANDA: Kampala, Jan. 31, 1930, *Hansford 1043*, Kabale, Kigezi, Aug. 1937, *Hansford 2230*.

Teliospores are present in No. 1043 and agree in all respects with those of *U. leptodermus*. It is probable that all specimens cited above belong to this species.

UROMYCES PRETORIENSIS Doidge. On *Aneilema* sp., UGANDA: West Nile, June 1936, *A. S. Thomas* (Hansford No. 1994); on *Commelina vogelii*, GOLD COAST: Aburi, Sept. 2, 1937, *Deighton CB960*.

The urediospores described by Doidge are apparently amphispores since, in Deighton's collection, there also occur urediospores with a wall thickness of 1.5–2 μ. These spores occur in separate sori or occasionally in the sori which produce the thick-walled amphispores. The rust on *Aneilema* consists of amphisori and telia.

UROMYCES SCHINZIANUS P. Henn. (fig. 7). On *Bauhinia fassoglensis*, UGANDA: Bukwa, Elgon, Dec. 1933, *Hansford 1699*.

The Sydows (Ann. Myc. **6**: 136. 1908) have reported that Hennings' description was based upon the *Uredo* form and that he incorrectly described the urediospores as being teliospores. The Sydows' description of the urediospores corresponds so closely to those in Hansford's specimen that I feel confident that the rusts are the same and, since telia are present, a complete description is given below.

Uredia amphigenous but mainly hypophyllous, scattered or loosely grouped, round, 0.2–0.4 mm. diam., subepidermal, more or less pulverulent, yellowish brown or darker; urediospores globoid or broadly ellipsoid, 25–28 × 27–31 μ (Sydow: 20–29 × 22–32 μ); wall 3–3.5 μ thick at sides, nearly uniform or thickened apically to 5 μ, golden to nearly chestnut-brown, variably bilaminate, the outer layer more or less hygroscopic, subhyaline, often conspicuous in mature spores, moderately echinulate; pores 3–5, equatorial or sometimes slightly above, appearing scattered unless the spore is properly oriented. Telia like the uredia or slightly larger and deep chestnut-brown, pulverulent; teliospores ovate or ellipsoid, rounded at the base and rounded or narrowly rounded at the apex, 18–23 × 23–31 μ; wall chestnut-brown, 3–4 μ thick at sides, 5–10 μ at the apex which is usually paler, finely reticulate with meshes 0.5–1 μ diam.; pedicel hyaline, fragile, about equal to the spore in length but often broken near the spore.

Uromyces aspiliicola Cummins, sp. nov. (fig. 9). Pycniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, in maculis minutis brunneis sparsis, rotundatis, 0.1–0.3 mm. diam., pulverulentis; urediosporis obovatis vel triangularis, 18–23 μ latis, 19–23 μ altis; membrana 1–1.5 μ cr., pallide cinnamomeo-brunnea, minuteque echinulata; poris germ. 2, aequatorialibus vel plus minusve subaequatorialibus. Teliis urediis conformibus sed atro-brunneis; teliosporae globoideae, 25–29 × 27–30 μ; membrana 4–6 μ cr., supra poro germ. umbono subhyalino leniter incrassata, minuteque rugosa vel fere levibus; pedicello hyalino, fragili, sporam breviore.

On *Aspilia asperifolia*, UGANDA: Kabaroni, Elgon, Dec. 1923, *Hansford 1723* (TYPE).

Uromyces baccarinii Syd. appears, from the description, to be a generally similar species but with verrucose teliospores having long, semipersistent pedicels.

PUCCINIA ANDROPOGONICOLA Har. and Pat. (fig. 3). On *Cymbopogon giganteus*, GOLD COAST: Accra Plains, Aug. 5, 1937, *Deighton CB854;* on *Cymbopogon* sp., UGANDA: Serere, Teso, Apr. 1933, *Hansford 1632;* on *Hyparrhenia rufa*, MAURITIUS: Riche terre, Sept. 6, 1939, *Wiehe 170;* on *Hyparrhenia* sp., UGANDA: Serere, Teso, Jan. 1933, *Hansford 1620.*

All specimens cited above have both uredia and telia and agree in essential features with *P. andropogonicola*, the type of which has been available for comparison. The species has larger teliospores with thicker apex than do most species on Andropogoneae. In the uredial stage it is perhaps not separable with accuracy from *P. nakanishikii* Diet. and *P. kaernbachii* (P. Henn.) Arth. *P. andropogonicola* was described from French Congo.

PUCCINIA NAKANISHIKII Diet. On *Cymbopogon excavatus*, UGANDA: Kinkizi, Kigezi, Aug. 1937, *Hansford 2149;* on *Cymbopogon martini*, UGANDA: Kawanda, Mar. 1941, Nov. 1942, *Hansford 2988, 3101;* on *Cymbopogon* sp., UGANDA: Serere, Teso, Mar. 1933, *Hansford 1626.*

Telia are present on Hansford's No. 2988, the teliospores deep chestnut-brown with persistent, brown pedicels approximately equaling the spores in length. I have had for comparison two specimens, both on *C. confertiflorus*, one from Belgian Congo and one from Ceylon.

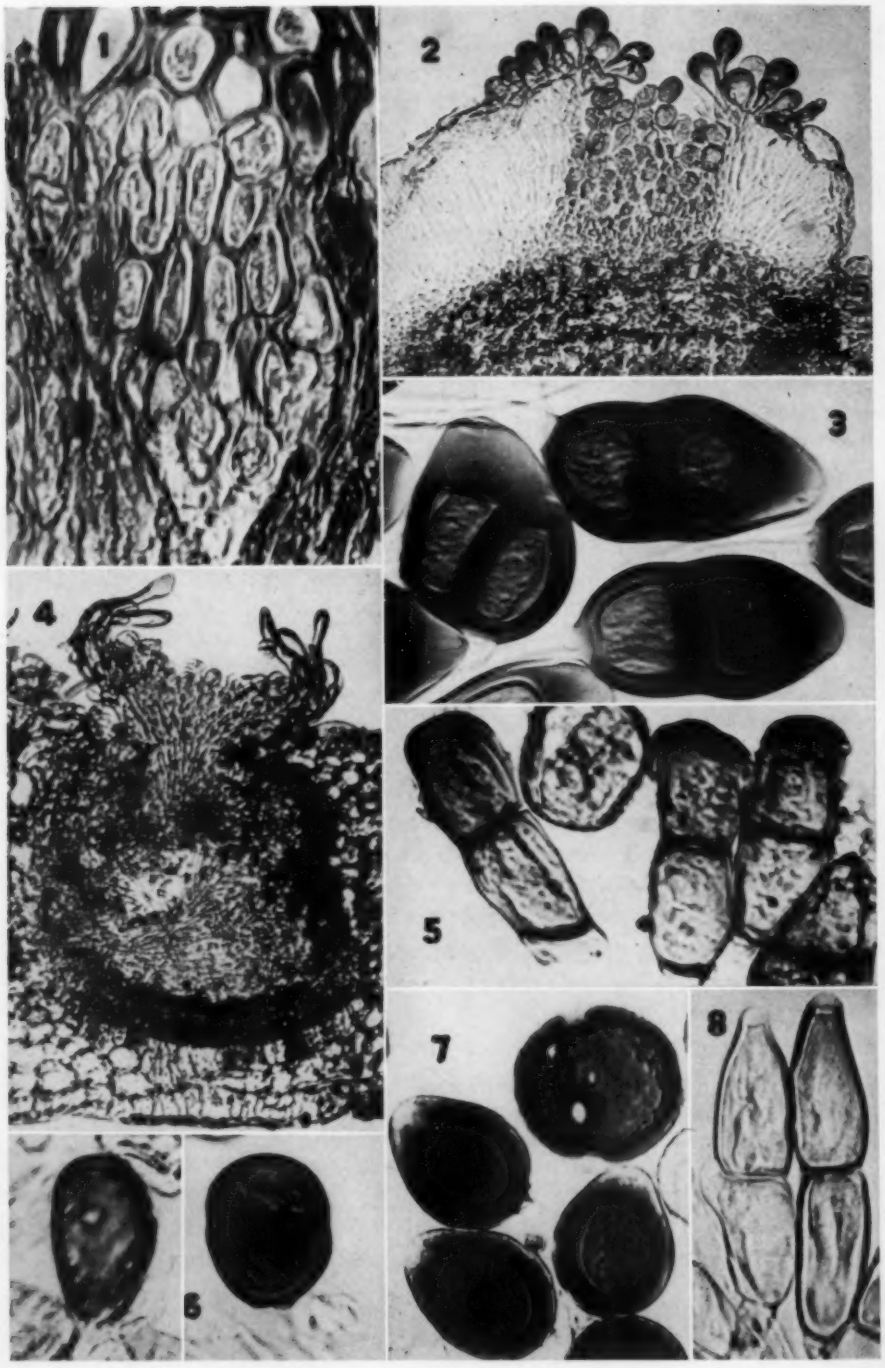
PUCCINIA INCOMPLETA Syd.? On *Andropogon dummeri*, UGANDA: Kawanda, Mar. 1941, *Hansford 2990.*

P. incompleta was described from India on *Ischaemum* and has been reported from Okinawa Island and Formosa by Hiratsuka. Sydow described the paraphyses as strongly incurved with the wall much thicker on the convex than on the concave side. Hansford's specimen agrees in this respect but has slightly larger spores, 17–23 × 25–30 μ as against 16–20 × 20–26 μ.

Sydow did not describe telia but reports the teliospores as "perpaucis tantum visi, 1-septatis, adhuc immaturis." The uredia in Hansford's specimen and those described by Sydow possess characteristics common in *Angiopsora* or *Phakopsora* and the rust may be found to belong in one of these genera. The genus *Angiopsora* had not been recognized when Sydow described *P. incompleta* and *Phakopsora* had not been reported as occurring on grasses. It should be noted that Sydow made no mention of pedicels on the teliospores.

PUCCINIA PROPINQUA Syd. On *Amphilophis insculpta*, UGANDA: Butiaba, 1933, *H. B. Johnston* (comm. *Hansford 2246*). This species has not been reported from Africa. It was described from India on *Andropogon* and differs from *P. cesatii* Schroet. in having somewhat smaller urediospores.

Puccinia kenmorensis Cummins, sp. nov. (fig. 6). Pycniis et aeciis ignotis. Urediis amphigenis, in maculis brunneis sparsis vel laxe aggregatis,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ovalis, usque ad 0.5 mm. longis, brunneis, pulverulentis; paraphysibus plerumque capitatis, rectis, incurvatis vel geniculatis, 11–16 × 50–75 μ; membrana pallide flavida vel aurea, 2–3 μ cr., ad apicem usque ad 8 μ cr.; urediosporae globoideae, late ellipsoideae vel ellipsoideae, 19–23 × 23–29 μ; membrana uniformiter 2–2.5 μ cr., obscure cinnamomeo- vel castaneo-brunnea, minuteque echinulata; poris germ. 6–8 sparsis. Teliis ignotis; teliosporae ellipsoideae vel late ellipsoideae, utrinque rotundatae, medio non vel vix constrictae, 18–22 × 23–30 μ; membrana 2–2.5 μ cr., castaneo- vel pallide castaneo-brunnea, levi; pedicello brevi, hyalino, fragili, haud raro oblique inserto.

On *Bothriochloa decipiens*, AUSTRALIA: Kenmore, near Brisbane, May 18, 1943, *Clemens* (TYPE); Imbil, Queensland, Dec. 26, 1943, *Clemens*.

The location of the germ pores in the teliospores is uncertain but the presence of pale areas in the wall indicate that the pore of the upper cell is apical while that of the lower is in the lower half of the cell. The pedicels of the teliospores are fragile and deciduous. The urediospores and teliospores are both approximately chestnut-brown.

The combination of characters exhibited by this rust distinguish it from species described as having paraphyses and echinulate urediospores with scattered pores. Perhaps *Puccinia andropogonis-micranthi* Diet. is the most similar species but it has larger teliospores with longer pedicels. The number and arrangement of the pores is not given in the description of *P. andropogonis-micranthi* Diet. and no specimen has been available for examination.

PUCCINIA CACAO McAlp. On *Hemarthria uncinata*, AUSTRALIA: Imbil, Queensland, Dec. 27, 1943, *Clemens*.

Teliospores, present in the uredia, agree in all respects with *P. cacao* as described and illustrated by McAlpine. Although paraphyses were not described for the uredia of *P. cacao* they are present in the Clemens' specimen and are like those described for *Uredo mira* Cumm. (Bull. Torrey Club **70**: 528. 1943) on *Manisuris altissima* from Argentina. While I have not seen type material of *P. cacao* I am confident that *U. mira* should be considered as a synonym.

Puccinia paspalina Cummins, sp. nov. (fig. 10). Pycniis et aeciis ignotis. Urediis amphigenis vel epiphyllis vel in vaginis culmisque evolutis, ovalis vel

Explanation of figures 1–8

FIG. 1. Freehand, unstained section of a telium of *Phakopsora grewiae*. × 800. FIG. 2. Freehand, unstained section of an uredium of *Ravenelia maranguensis* showing spores, brown peripheral paraphyses and adjacent gelatinous material. (From Hansford *1737*.) × 170. FIG. 3. Teliospores of *Puccinia andropogonicola*. (From Hansford *1632*.) × 800. FIG. 4. Freehand, unstained section of a sorus of *Uredo corbiculoides*. The sori characteristically arise in the sunken areas of the leaf. (From type.) × 170. FIG. 5. Teliospores of *Puccinia dolosoides*. (From type.) × 800. FIG. 6. *Puccinia kenmorensis;* urediospore and teliospore. (From type.) × 800. FIG. 7. Teliospores and one urediospore of *Uromyces schinzianus*. (From Hansford *1699*.) × 800. FIG. 8. Teliospores of *Puccinia kampalensis*. (From type.) × 800.

oblongis, 0.3–0.8 mm. longis, sparsis, pulverulentis, brunneis; paraphysibus nullis; urediosporae ovatae, obovoideae, ellipsoideae, frequenter plus minusve angulatae, 19–26 × 25–35 μ; membrana 1.5 μ cr., cinnamomeo- vel pallide cinnamomeo-brunnea, subtiliter breviterque echinulata; poris germ. 3, aequatorialibus. Teliis urediis similibus sed tarde dehiscentibus; teliosporae clavatae, variabiles, ad apicem obtusae vel rotundatae, deorsum attenuatae, medio non vel vix constrictae, 23–30 × 38–46 μ; membrana 1.5–3 μ cr., ad apicem non vel vix inerassata, flavida vel pallide aureo-brunnea, levi; pedicello persistenti, flavidulo, 6–10 μ longo; mesosporis numerosis.

On *Paspalum orbiculare*, AUSTRALIA: Birkdale, near Brisbane, Apr. 19, 1943, *Clemens* (TYPE); Brisbane, Mar. 4, 1943, Apr. 26, 1943, *Clemens;* on *P. dilatatum*, Mt. Coot-tha, near Brisbane, Feb. 24, 1943, *Clemens*.

It is probable that *Uredo paspalina* Syd. represents the uredial stage of this species but I have not had authentic material for comparison. If this assumption is true the rust may be rather widely distributed in the islands of the Pacific.

The long-covered telia, the pale irregular teliospores, the presence of numerous mesospores, and the lack of a definitely thickened apical wall clearly distinguish *P. paspalina* from *P. substriata* and other species of *Puccinia* described on *Paspalum*. Telia, present only in the type specimen and scantily in it, were found with certainty only on the leaf sheaths.

Puccinia dolosoides Cummins, sp. nov. (fig. 5). Urediis hypophyllis, sparsis, ellipticis, 0.2–0.5 mm. longis, flavo-brunneis, epidermide rupta inconspicue; paraphysibus nullis; urediosporae ovatae vel late ellipsoideae, 21–27 × 27–33 (–36) μ; membrana 1.5 μ cr., moderate echinulata, pallide cinnamomeo-brunnea; poris germ. 3 (vel 4) equatorialibus. Teliis hypophyllis, subepidermalibus, indehissentibus, sparsis vel laxe aggregatis, atrobrunneis, oblongis, usque ad 0.7 mm. longis, 0.1 mm. latis; teliosporae plerumque clavatae, ad apicem late rotundatae vel obtusae, deorsum attenuatae, ad septum non vel leniter constrictae, (16–) 18–23 (–25) × 34–43 (–50) μ; membrana castaneo-brunnea, 1–1.5 μ cr. vel ad apicem 2–4 μ cr.; pedicello brunneolo, persistenti, brevi, 6–10 μ longo.

On *Paspalum commersonii*, SIERRA LEONE: Njala, Sept. 24, 1926, *F. C. Deighton 32* (TYPE).

This rust is similar, because of the small covered telia, to *P. dolosa* Arth. and Fromme and to *P. chaetochloae* Arth. In *P. dolosa* both the urediospores and the teliospores are smaller and the urediospores are triangular. *P. chaetochloae* has somewhat larger, more angular, and thicker-walled urediospores. The teliospores in Deighton's specimen are more regularly clavate than in *P. dolosa* or *P. chaetochloae*.

PUCCINIA SUBSTRIATA Ellis and Barth.? On *Solanum anomalum*, SIERRA LEONE: between Wiema and Dia, Apr. 6, 1936, *Deighton 999*.

In gross appearance and microscopic characteristics the aecia in this collection agree well with American collections of *P. substriata* (*Aecidium*

tubulosum). Inoculation experiments, using African material, will be necessary to prove the relationship since no African rust has yet been seen on *Paspalum* which can unquestionably be assigned to *P. substriata*.

Puccinia stenotaphri (Syd.) Cummins, comb. nov. (*Uredo stenotaphri* Syd., Ann. Myc. **7**: 544. 1909.) Uredia amphigenous but frequently mainly epiphyllous, scattered or in small groups and then usually on small yellowish spots, elliptical or oblong and attaining 0.5 mm. in length, pulverulent, light cinnamon-brown; paraphyses peripheral, in moderate numbers, slightly incurved, cylindrical, 9–12 × 50–75 μ; wall yellowish or nearly hyaline, 1.5–3 μ thick, the apex uniform or slightly thickened; urediospores broadly ellipsoid, ovoid or ellipsoid, (22–) 25–28 (–30) × (28–) 30–40 (–46) μ; wall 1.5 μ thick, light cinnamon or cinnamon-brown, moderately echinulate; pores 4 or 5, equatorial, distinct. Telia amphigenous or mainly hypophyllous, subepidermal, long-covered by the epidermis, blackish brown, scattered or in small groups, oblong, 75–110 μ wide and attaining a length of 1 mm.; teliospores somewhat variable but mainly clavate or oblong-clavate, the apex obtuse-rounded, slightly or not constricted at the septum, narrowed toward the pedicel, 19–26 × (37–) 44–60 μ; wall chestnut-brown, 1.5 μ thick at sides, thickened apically to 2.5–4 μ, smooth; pedicel brownish, persistent, 6–12 μ long.

On *Stenotaphrum dimidiatum*, MAURITIUS: June 1913, *Wiehe 115*.

The Syndows (*l.c.*) did not describe paraphyses in *Urego stenotaphri* and I have not had the type specimen for study, but I assume that the above transfer is valid. *P. stenotaphri* is similar to *P. penniseti* Zimm., a species in which paraphyses were not described nor are they present in material which I have had for comparison.

PUCCINIA SCLERIAE (Pazschke) Arth. On *Scleria naumanniana*, SIERRA LEONE: Njala, Nov. 7, 1938, *Deighton 1637*.

Only uredia are present but they agree with those of *P. scleriae*.

Puccinia kampalensis Cummins, sp. nov. (fig. 8). Pycniis et aeciis ignotis. Urediis hypophyllis vel rarius epiphyllis, sparsis, rotundatis, 0.2–0.3 mm. diam., pulverulentis, cinnamomeis vel obscure cinnamomeis; urediosporae late ellipsoideae vel ellipsoideae, 17–21 × 22–26 μ; membrana 1.5 μ cr., cinnamomeo-brunnea, moderate echinulata; poris germ. 2, aequatorialibus. Teliis urediis conformibus sed plus minusve pallidiore; teliosporae oblongae, apicem versus plerumque attenuatae, ad septum leniter constrictae, basim versus attenuatae, 12–17 (–19) × (40–) 48–65 μ; membrana 1 μ cr., ad apicem 2–3 μ cr., pallide aureo-brunnea, levi; pedicello hyalino, usque 40 μ longo.

On *Wedelia oblonga*, UGANDA: Kampala, Feb. 1937, *Hansford 1073* (TYPE). *P. kampalensis* is similar to *P. caracasana* Syd., known only from Venezuela, but differs in having only two germ pores in the urediospores.

PUCCINIA MINUSSENSIS Thüm., var. **africana** Cummins, var. nov. Pycnia nulla. Aecia hypophylla et caulicola, peridio destituta, poro centrali aperta, 0.3–0.5 mm. diam., per totam superficiem plus minusve aequaliter sparsa;

aeciosporae globoideae vel ellipsoideae, 14–20 × 18–26 μ; membrana 1.5 μ cr. verruculosa. Urediis hypophyllis, sparsis, 0.1–0.3 mm. diam., cinnamomeo-brunneis; urediosporae globoideae vel late ellipsoideae, 18–20 × 19–24 (–26) μ; membrana 1.5–2 μ cr., echinulata; poris germ. plerumque 3, aequatorialibus vel 3–4 plus minusve sparsis. Teliis urediis conformibus sed castaneis; teliosporae ellipsoideae, 22–28 × 29–35 μ; membrana 2–2.5 μ cr., castaneo-brunnea, verrucosa; poro superiore apicali, inferiore infra medium loculum sito; pedicello hyalino, fragili.

On *Lactuca* sp., UGANDA: Lwasamaire, Ankole, Aug. 1937, *Hansford 2156;* Kabale, Kigezi, Aug. 1937, *Hansford 2211* (TYPE).

This variety differs from the typical *P. minussensis* in that pycnia are lacking, the aecia are smaller and the urediospores usually have three equatorial pores. Aecia and uredia are present in No. 2156, the uredia on separate leaves or scattered among the aecia, while uredia and telia are present in the type.

Sphenospora mera Cummins, sp. nov. Pycniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, sparsis, rotundatis, usque ad 0.5 mm. diam., cinnamomeo-brunneis; urediosporae ovoideae vel late ellipsoideae, 17–22 × 23–27 μ; membrana 2–2.5 μ cr., cinnamomeo- vel aureo-brunnea, moderate echinulata; poris germ. 3 vel 4, aequatorialibus. Teliis hypophyllis, subepidermalibus, epidermide rupta inconspicue, in greges 1–2 mm. diam. concentrice dispositis, ceraceis, aureo-brunneis, plus minusve pulvinatis; teliosporae longitudinaliter uniseptatae, ad septum non constrictae, oblongo-ellipsoideae, 11–14 × 20–26 μ; membrana hyalina, 0.5 μ cr.; pedicello persistenti, 9–12 × 30–45 μ, hyalino.

On *Cycnoches chlorochilon*, VENEZUELA: locality unknown; intercepted in customs at San Juan, Puerto Rico, Nov. 16, 1943, by *L. J. McConnell 8719* (TYPE). Type deposited in the Arthur Herbarium; the Mycological Collections of the U. S. Department of Agriculture; and the Pennsylvania State College Herbarium.

This is the second species of *Sphenospora* to be collected on the Orchidaceae.

Ravenelia hansfordii Cummins, sp. nov. Pycniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, sparsis, rotundatis, usque 0.4 mm. diam., fusco-brunneis; periphysibus copiosis, cylindraceis vel plus minusve clavatis, plerumque 1-septatis, brunneis vel deorsum hyalinis, 8–14 × 50–65 μ; membrana 1 μ cr., ad apicem 2–4 μ cr.; urediosporae ellipsoideae vel oblongae, 14–18 × 28–38 μ; membrana 1–1.5 μ cr., aureo- vel pallide cinnamomeo-brunnea, minuteque echinulata; poris germ. 4 (vel 5), aequatorialibus vel plus minusve subaequatorialibus. Teliis amphigenis, subepidermalibus, sparsis, atro-brunneis; capitulis teliosporarum convexis, castaneo-brunneis, 65–99 μ diam., ex sporis 5–7 in omni directione compositis; sporis singulis unicellularibus, 13–20 μ diam., papillis verruciformibus 6–10 minutis 0.5–1.5 μ altis obsitis; membrana 0.5–1 μ cr., ad apicem 4–6 μ cr.; cystidiis numerosis, globosis, pendulis, eodem numero quo sporis singulis, in aqua facile diffluentibus; pedicello ex hyphis pluribus composito, deciduo.

On *Acacia* sp., UGANDA: Serere, Teso, Jan. 1933, *Hansford 1616* (TYPE).

R. hansfordii differs from species described previously on *Acacia* because of longer urediospores, the low wart-like markings on the teliospores and especially because of the usually septate, puccinioid paraphyses.

RAVENELIA BOTTOMLEYAE Doidge. On *Albizzia grandibracteata*, UGANDA: Kawanda, July 1939, *Hansford 2524*.

The aecial stage, not previously described, is abundant on Hansford's specimen. Pycnia amphigenous, lenticular, 150–175 μ diam., subcuticular in origin but extending downward between and partially displacing the epidermal cells, aparaphysate, hymenium flat. Aecia uredinoid, occurring as a more or less complete ring about the pycnia, 2–5 mm. diam., usually associated with the veins, reddish brown, more numerous on the upper leaf surface; aeciospores asymmetrical, ovate, obovate or ellipsoid with pores face-view, 22–25 × 29–36 μ, mainly oblong with pores lateral, 16–20 × 29–36 μ; wall laminate, the inner wall golden-brown, 1.5 μ thick, the outer wall hyaline or yellowish, 2–6 μ thick, investing the spore as a simple band, appearing as a more or less complete envelop when pores are face-view but as a conspicuous apical and slight basal cap when pores are lateral, rather closely and strongly aculeate with conical spines, smooth on the pore-bearing sides; pores 2, equatorial.

Doidge's description gives the thickness but does not mention the character of the wall of the urediospores, which is similar in structure to that of the aeciospores.

RAVENELIA LAEVIOIDES Arth. and Cumm. On *Indigofera* sp., UGANDA: Serere, Teso, Oct. 1932, *Hansford 1601;* Kinkizi, Kigezi, Aug. 1937, *Hansford 2153*.

This species was described from the Philippine Islands and has not been reported from other regions. Both uredia and telia are represented in Hansford's collection and agree morphologically with *R. laevioides*. In the type specimen the telia are caulicolous whereas they are foliicolous in the African material.

Ravenelia maranguensis (P. Henn.) Cummins, comb. nov. (fig. 2) (*Uredo maranguensis* P. Henn. in Engler, Die Pflanzenwelt Ost-Africas und der Nachbargebiete, Teil C, p. 51, 1895). Uredia hypophyllous, subepidermal, usually in compact groups 1–3 mm. diam. on yellowish spots, pulvinate, round, 0.3–0.7 mm. diam., cinnamon-brown or becoming darker, opening at first by a narrow aperture surrounded by paraphyses and an extensive adjacent, gelatinous, subepidermal layer which apparently produces the paraphyses and is in turn dissolved as the sorus develops; paraphyses mainly capitate or clavate-capitate, 14–26 μ diam., the wall golden to chestnut, 3–9 μ thick at the apex; urediospores broadly ellipsoid or ellipsoid, 16–18 × 18–25 μ; wall 1.5–2 μ thick, pale yellowish or nearly hyaline, rather finely and closely echinulate; germ pores 6–9, scattered. Telia amphigenous but mainly epiphyllous and opposite the uredia, blackish brown; teliospore-heads convex, 80–135 (–140) μ diam., about 35 μ thick, chestnut brown, the marginal spores bearing 1–6, usually 1–3, subhyaline, papillate tubercles 1–4 μ high; indi-

vidual spores 2-celled except the marginal row, 13–23 μ diam.; wall 1.5–2 μ thick at sides, 4–6 μ at apex; cysts hyaline, appressed, equal in number to the marginal cells, swelling and bursting in water; pedicel hyaline, composed of many hyphae, easily broken.

On *Indigofera arrecta*, UGANDA: Kisoro, Kigezi, Aug. 1937, *Hansford 2197;* on *Indigofera* sp., UGANDA: Butandiga, Mt. Elgon, Aug. 1934, *Hansford 1737.*

Both uredia and telia are present in No. 1737, from which the above description is drawn. I have had for study a specimen, probably part of the original collection, of *Uredo maranguensis* on *Indigofera arrecta* collected by G. Volkens at Marangu, Kilimandscharo in 1894. The sori are older and the gelatinous layer less conspicuous but the rusts are unquestionably the same. Because of the characteristic structure of the uredia, *R. maranguensis* is readily distinguishable from other species on *Indigofera.*

SPHAEROPHRAGMIUM ACACIAE (Cooke) Magn. On *Albizzia lebbek*, GOLD COAST: Aburi Bot. Gard., July 27, 1937, *Deighton CB827;* RODRIGUIS ISLAND: Oct. 1938, *Wiehe DR12;* on *Albizzia procera*, GOLD COAST: Aburi Bot. Gard., July 27, 1937, *Deighton CB826;* on *Cassia* sp., UGANDA: Serere, Teso, Jan. 1933, *Hansford 1622.*

It was impossible to find differences, either in the uredia or the telia, between the rust on *Albizzia* and on *Cassia* and they are considered to represent a single species.

Phragmopyxis leonensis Cummins, sp. nov. (fig. 11). Pycniis et aeciis ignotis. Uredia hypophylla, sparsa vel in maculis brunneis usque 2.5 mm. diam. aggregata, rotundata usque 0.3 mm. diam., flavida, subcuticularia; periphysibus copiosis, hyalinis vel brunneis, cylindraceis, ad basim conjunctis et septatis, crasse tunicatis, fere solidis, 10–18 × 42–85 μ; urediosporae late ellipsoideae vel ellipsoideae, 13–16 × 16–19 μ; membrana 1–1.5 μ cr., hyalina vel subhyalina, minuteque echinulata, poris germ. obscuris, sparsis, plus minusve 8. Teliis urediis conformibus sed castaneis; teliosporae cylindraceae, utrinque rotundatae, ex cellulis 2–3, plerumque 3, compositis, 19–23 × (37–) 42–50 (–54) μ; membrana bilaminata, pariete interiore aureo-vel pallide castaneo-brunnea 2 μ cr., exteriore subhyalina usque 4 μ cr., valde tuberculato-aculeata, aculeae usque 3 μ lata, 5 μ alta; pedicello sporam aequante vel longiore, hyalino, persistenti. Statim germ.

On *Cassia sieberiana*, SIERRA LEONE: Njala, Nov. 30, 1934, *Deighton 660* (TYPE).

This unusual rust is probably best referred to the genus *Phragmopyxis*, although in certain features it approaches the genus *Mimema*, established by Jackson (Mycologia **23**: 338. 1931) to accommodate a species (*M. holwayi* Jacks.) on *Cassia* from Bolivia. In *Mimema* the sori are subepidermal with peripheral paraphyses and the teliospores cylindric with a uniformly thin, nonlaminate wall and a long, nearly solid pedicel. The genus is strongly reminiscent of *Hamaspora*, as pointed out by Jackson.

In *Phragmopyxis* the sori, other than pycnia, are likewise subepidermal with peripheral, but less strongly developed, paraphyses. The teliospores are phragmidioid with a conspicuously laminate wall, the outer hyaline portion hygroscopic, and pedicels which are nearly solid near the spore but delicate and evanescent or strongly hygroscopic in the lower portion. There are three or four equatorial pores in each cell of the teliospore in *Phragmopyxis*. In *Mimema* pores are not obvious but the basidium is produced at the apex of the upper cell and just below the septum in the other cells.

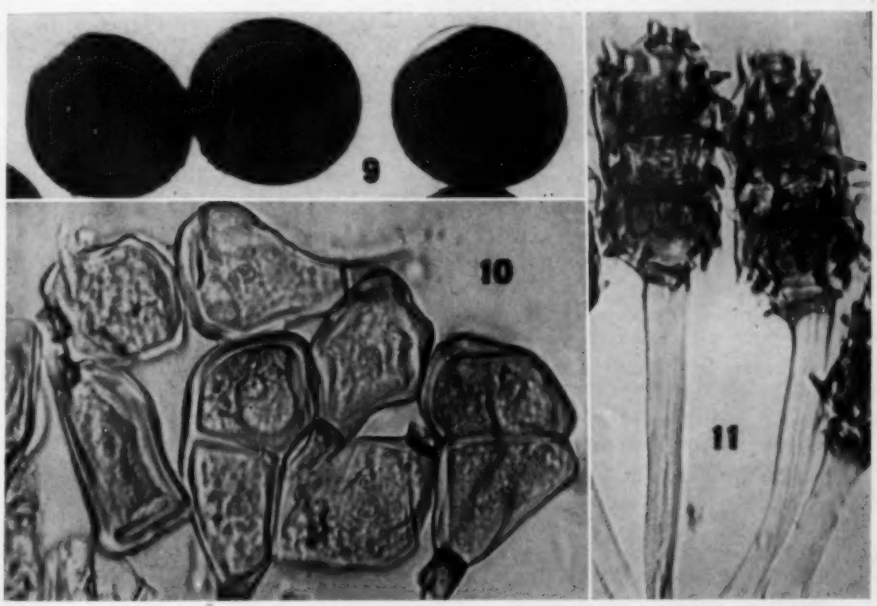

FIG. 9. Teliospores of *Uromyces aspiliicola*. The wall is dark brown except for a slight hyaline umbo over the pore. (From type.) × 800. FIG. 10. Teliospores of *Puccinia paspalina*. One-celled teliospores are common. (From type.) × 800. FIG. 11. Teliospores of *Phragmopyxis leonensis*. (From type.) × 800.

In Deighton's specimen the subcuticular position of the sori is not in conformity with either *Mimema* or *Phragmopyxis*. The large paraphyses are more like those in *Mimema*, but this is mainly a size similarity, and the urediospores offer no special features. The aculeate teliospores with their obviously laminate walls indicate relationship with *Phragmopyxis* but offer no similarity to *Mimema*. Germ pores have not been observed in Deighton's rust. There is, around the equator of each cell, a hyaline, smooth zone from which the basidium is produced but I have been unable to ascertain whether the basidium emerges from differentiated pores or whether the entire zone is equally susceptible to rupture during germination.

While recognizing that certain characteristics of this rust prevent unobjectionable assignment to any established genus, the number of characters in agreement with *Phragmopyxis* indicate that the species can be placed most logically in that genus.

Pucciniosira deightonii Cummins, sp. nov. Pycniis nullis. Teliis hypophyllis, in maculis brunneis usque 8 mm. diam. aggregatis, flavidis, aecidioideis, breviter cylindraceis; cellulis peridii plerumque oblongis, 13–20 × 24–44 μ; membrana 3 μ cr., minuteque verrucosa; teliosporis bicellularis plerumque ellipsoideis vel oblongo-ellipsoideis, (13–) 15–20 (–23) × (19–) 22–29 μ; membrana 1–1.5 μ cr., pallide flavidula vel hyalina, minuteque verruculosa.

On *Jasminum pauciflorum*, SIERRA LEONE: Hill Station, May 25, 1935, *Deighton 717* (TYPE); Njala, May 19, 1934, *Deighton 654A;* Allen Town, Dec. 7, 1936, *Deighton 1133.*

The infections vary in that some cause somewhat corky, discoid hypertrophy while in others this is not obvious. Hypertrophy appears to be commonest in older infections in which the telia have largely disintegrated.

Uredo arundinellae-nepalensis Cummins, sp. nov. Urediis amphigenis, in maculis brunneis aggregatis vel sparsis, ovalis vel oblongis, 0.2–0.6 mm. longis, brunneis, pulverulentis; periphysibus cylindraceis vel plus minusve incurvatis, 9–12 × 35–45 μ, membrana hyalina vel flavidula, 1–1.5 μ cr., ad apicem usque 6 μ cr.; urediosporae ellipsoideae, ovoideae vel obovoideae, 17–23 × 25–33 (–39) μ, membrana 1–1.5 μ cr., subtiliter echinulata, flavida vel aureo-brunnea; poris germ. inconspicuis, 4 vel 5, aequatorialibus.

On *Arundinella nepalensis*, AUSTRALIA: Lawnton, near Brisbane, Apr. 26, 1943, *Clemens;* Mt. Coot-tha, near Brisbane, February 24, 1943, *Clemens* (TYPE).

No rust previously reported on *Arundinella* is described as having paraphyses or urediospores with such thin walls. The paraphyses are usually thickened apically and dorsally and are similar, as are also the pale, thin-walled urediospores, to those of species of *Phakopsora* and *Angiopsora.*

Uredo crassiperidiata Cummins, sp. nov. Urediis amphigenis, supepidermalibus, sparsis, fusco-brunneis, pulverulentis, ovalis, 0.2–0.35 mm. longis, poro centrali apertis, peridio crasso, ex cellulis elongatis brunneis composito, membrana 2 μ crassa; urediosporae variabiles ellipsoideae, oblongo-ellipsoideae, obovatae vel late ellipsoideae, 18–23 (–26) × (25–) 29–35 (–39) μ; membrana 1.5–2 μ cr., hyalina vel pallide flavida, tenuiter laxiusculeque echinulata (2–2.5 μ): poris germ. 3 vel 4 aequatorialibus parum conspicuis praeditis.

On *Scleria melanomphala*, SIERRA LEONE: Segbwema, Dec. 11, 1937, *Deighton 1482* (TYPE).

The peridium provides the principal distinguishing feature of this species. It is 3–8 cells in thickness in the basal portion and bears some resemblance to the subepidermal paraphyses found in certain loculate telia. The

peridial tissue extends beneath the sorus, where its upper layer becomes sporogenous. At the sides, and especially toward the apex, some of the cells of the inner layer extend inward as short, variable paraphyses. The urediospores are longer than those of other rusts of *Scleria*.

Uredo achyranthicola Cummins, sp. nov. Urediis plerumque epiphyllis, aggregatis vel sparsis, rotundatis, 0.2–0.4 (–0.7) mm. diam., pulverulentis, flavidis, epidermide rupta conspicue; urediosporae obovoideae, ellipsoideae vel late ellipsoideae, (13–) 15–18 × (19–) 21–26 (–29) μ; membrana 2–2.5 μ cr., moderate echinulata, pallide aurea vel flavida; poris germ. obscuris, verissimiliter plus minusve 6, sparsis.

On *Achyranthes aspera*, UGANDA: Kisoro, Kigezi, Aug. 1937, *Hansford 2194* (TYPE).

In 1937 Castellani and Ciferri (Prodomus Mycofl. Afr. Orient. Ital. p. 52) reported *Uredo achyranthis* P. Henn. on *Achyranthes aspera* although the Sydows (Monogr. Ured. **4**: 591. 1924) state that Hennings' fungus is an *Albugo* and not a rust. Hansford's collection demonstrates that there is an *Uredo* on *Achyranthes* in Africa and, since Hennings' species is not a rust and since the *Uredo* differs from other rusts on Amaranthaceae, the above description is necessary.

Uredo diocleae Cummins, sp. nov. Urediis subepidermalibus hypophyllis, sparsis, pulverulentis, cinnamomeis, rotundatis, 100–175 μ diam.; urediosporae asymmetricae, globoideae vel late ellipsoideae, 19–23 × 22–26 μ, vel reniformae, 16–19 × 22–26 μ; membrana 1.5 μ cr., cinnamomeo-brunnea, moderate echinulata; poris germ. 1 (vel 2?) plus minusve superaequatorialibus.

On *Dioclea reflexa*, SIERRA LEONE: Mamanka, Feb. 1, 1939, *Deighton 1815* (TYPE).

Asymmetrical urediospores are not uncommon in rusts parasitic on legumes but are especially characteristic of the genus *Sphaerophragmium*. Only one pore, located slightly above the equator in the concave side of the spore, was seen with certainty but it is possible that there are two. An area surrounding the pore and extending down the concave side to the hilem is smooth, while other portions of the wall are uniformly echinulate.

UREDO FAMELICA Arth. and Cumm. On *Mucuna urens*, SIERRA LEONE: Kenema, Dec. 5, 1937, *Deighton 1438*.

The spores in this collection average slightly smaller than those of the type, collected in Ecuador, but do not differ otherwise.

Uredo corbiculoides Cummins, sp. nov. (fig. 4). Urediis hypophyllis, subepidermalibus, in lacunae foliorum natis, sparsis vel laxe aggregatis, brunneis, plus minusve corbiculaeformibus; paraphysibus peripherales copiosis, incurvatis, brunneis, 6–13 × 50–100 μ, deorsum conjunctis, mem-

brana 1–2 μ cr., superne incrassato usque ad 4 μ; urediosporae late ellipsoideae vel globoideae, 13–16 × 16–20 μ; membrana hyalina vel pallide aureo-brunnea, laxe breviterque echinulata; poris germ. obscuris.

On *Grewia* sp., UGANDA: Serere, Teso, Mar. 1933, *Hansford 1630* (TYPE).

This species has peculiar sori with abundant and conspicuous peripheral paraphyses which arise from an elongate, cellular, basal, stipe-like structure which arises from within the sunken areas which are characteristic of the leaves of the host. The spores are also produced from the apical layers of this cellular base and are sessile or nearly so. Their exact method of development was not ascertained.

U. corbiculoides differs from *Uredo grewiae* Pat. and Har. in having spores that are smaller (13–16 × 16–20 μ as against 15–21 × 20–28 μ) and paraphyses that are twice as long (50–100 μ as against 25–45 μ) as described for *U. grewiae*.

UREDO LONGAENSIS P. Henn.? On *Combretum* sp., GOLD COAST: Akwadum, Aug. 17, 1937, *Deighton CB908;* SIERRA LEONE: Mabang, Nov. 27, 1936, *Deighton 1346*.

It is questionable whether these collections are correctly referred to *U. longaensis* or whether they represent a related, but distinct, species. The sori are similar in habit, the spores are catenulate and of the same size but the wall is thickened apically in most spores, frequently to as much as 6 μ. The outermost layer of spores adheres to the epidermis in a peridium-like manner. These spores (or peridial cells?) are globoid, 13–16 μ in diameter and have walls of uniform thickness.

Uredo pergulariae Cummins, sp. nov. Urediis subepidermalibus, hypophyllis, sparsis vel laxe aggregatis, rotundatis, 0.3–1 mm. diam., pulverulentis, cinnamomeo-brunneis; urediosporae late ellipsoideae vel globoideae, 20–26 × 23–29 μ; membrana cinnamomeo-brunnea, 1.5–2.5 μ cr., moderate echinulata; poris germ. 4 (vel 3), aequatorialibus vel interdum plus minusve sparsis.

On *Pergularia* sp., UGANDA: Rifle Range, Kiagwe, June 1942, *Hansford 3041* (TYPE).

Uredo perscita Cummins, sp. nov. Urediis hypophyllis, subepidermalibus, sparsis vel laxe aggregatis, pulverulentis, flavo-brunneis, rotundatis, 0.2–0.4 mm. diam.; paraphysibus peripherales copiosis, plerumque 1-septatis, incurvatis, inferne conjunctis, 13–21 μ latis et usque 80 μ longis, flavidis vel plerumque brunneis, deorsum pallidiore, membrano ubique 1.5–2.5 μ cr.; urediosporae ovate vel ellipsoideae, 21–23 (–26) × (26–) 29–35 (–40) μ; membrana 1–1.5 μ cr., hyalina vel pallide flavida, moderate echinulata; poris germ. obscuris.

On *Vernonia amygdalina,* UGANDA: Rukingiri, Kigezi, Aug. 1937, *Hansford 2171* (TYPE).

This species is undoubtedly related to *Uredo vernoniicola* Petch and *U. toroiana* Kern, both also on *Vernonia,* but differs because of larger urediospores.

Uredo palpigera Cummins, sp. nov. Urediis subepidermalibus, epiphyllis rarius hypophyllis, brunneis, in maculis brunneis sparsis vel laxe aggregatis, poro centrali apertis, rotundatis, 0.1–0.3 mm. diam.; paraphysibus plerumque peripheriicis, 1–2-septatis, plus minusve puccinioideis, cylindraceis vel clavatis, rectis vel incurvatis, deorsum conjunctis, hyalinis vel brunneis, 10–18 × 45–115 μ; membrana 1–2 μ cr. vel ad apicem usque 8 μ cr.; urediosporae late ellipsoideae vel ellipsoideae, 17–23 × 25–29 μ; membrana 1.5–2 μ cr., flavidula vel pallide flavo-brunnea, moderate tenuiter echinulata; poris germ. obscuris.

On *Inula acervata*, UGANDA: Kigezi, Aug. 1937, *Hansford 2150* (TYPE).

Uredo brachylaenae Doidge, which occurs on the genus *Brachylaena* of the tribe Inuleae, is probably similar but has smaller spores (15–17 × 18–23.5 μ). These two species are morphologically similar to *U. perscita, U. toroiana* and *U. vernoniicola* and perhaps all will be found to belong in the genus *Phakopsora*. Hiratsuka (Bot. Mag. Tokyo **49**: 854. 1935) has recently transferred *Uredo elephantopodis*, a species with similar morphology, to *Phakopsora*.

Aecidium entebbense Cummins, sp. nov. Pycniis epiphyllis, aggregatis, brunneis, subcuticularibus, lenticularis 50–70 μ altis, 110–170 μ latis. Aeciis hypophyllis, subepidermalibus, in maculis pallide brunneis usque ad 1.5 cm. dense aggregatis, cupulatis, 100–150 μ diam., margine albido, recurvato; cellulis peridii firme conjunctis, oblongis vel polyhedricis 13–19 × 18–25 μ, pariete exteriore striato 3–3.5 μ cr., interiore verrucoso 2–3 μ cr.; aeciosporae globoideae, ellipsoideae vel oblongo-ellipsoideae, 13–18 × 16–23 μ; membrana 1 μ cr., ad apicem 3–8 μ cr., hyalina vel pallide flavida, verrueulosa.

On *Phyllanthus discoideus*, UGANDA: Entebbe Road, June 1942, *Hansford 3016* (TYPE).

None of the species of *Aecidium* previously described on *Phyllanthus* has aeciospores with an apically thickened wall. One of these, *A. phyllanthi* P. Henn., occurs on *P. discoideus* in Africa. In addition to having aeciospores with uniform walls, it differs also in having hypophyllous pycnia. The pycnia in *A. entebbense* have a flat hymenium and are bordered by peridial tissue composed of long, narrow cells, as is frequently true of subcuticular pycnia.

AECIDIUM EVANSII P. Henn. On *Lippia* sp., UGANDA: Sabei, Elgon, December 1933, *Hansford 1678*.

Certain morphological features have not been sufficiently emphasized in published descriptions of this species. The walls of the aeciospores vary in thickness because of invaginations of the inner surface. These invaginations are numerous, frequently pronounced and occur both in the side and the apically thickened areas of the wall. This character is exhibited also by a specimen on *L. asperifolia* collected by Pole Evans in the Pretoria District of Transvaal (Mycol. Herb. Union Dept. Agr. no. 5186; identified by Miss

Doidge), in a specimen on *L. berlandieri* of uncertain origin (Mexico?), and can be considered characteristic of the species. In Hansford's specimen the aecia are cylindric, about 1 mm. in length, and tend to develop along the veins of the leaf.

AECIDIUM VERNONIAE-PODOCOMAE Doidge. On *Vernonia* sp., UGANDA: Kawanda, Kampala, June 1939, *Hansford 2475*.

This rust has not been recorded for Uganda. The specimen was received as *? Aecidium vanderystianum* P. Henn. but has the thick-walled spores described by Doidge.

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NEW COMBINATIONS IN CORNUS

H. W. RICKETT

The following nomenclatural changes in *Cornus* are proposed for the sake of consistency in a forthcoming treatment of the genus in North American Flora.

CORNUS DRUMMONDI f. **priceae** (Small) Rickett, comb. nov. *C. priceae* Small, Torreya **1**: 54. 1901. This form differs from typical *C. drummondi* (*C. asperifolia* of authors) only in the small size of the fruit. It has been reported only from the type locality.

CORNUS FLORIDA subsp. **urbiniana** (Rose) Rickett, comb. nov. *C. urbiniana* Rose, Contr. U. S. Nat. Herb. **8**: 53. 1903. *C. florida* var. *urbiniana* Wangerin in Engler, Pflanzenreich IV. 229 (**41**): 87. 1910. The Mexican plants so designated differ from *C. florida* of the eastern United States in several minor characters: the narrow acute (not retuse) involucral bracts; fewer flowers in each inflorescence; usually only 1–3 fruits in a cluster. Such distinctions are scarcely of specific importance; were it not for the geographic disjunction, it would probably be impossible to segregate, on such bases, two independent populations. On the other hand, the two populations are at present perfectly distinct; and for this reason (as well as because of the rather anomalous status of the concept of varietas) it seems appropriate to consider them as subspecies.

CORNUS DISCIFLORA f. **floccosa** (Wangerin) Rickett, comb. nov. *C. floccosa* Wangerin, Repert. Sp. Nov. **6**: 101. 1908. *C. disciflora* var. *floccosa* Standl. Field Mus. Publ. Bot. **8**: 321. 1931. The leaves of typical *C. disciflora* are pubescent with very minute appressed forked trichomes. *C. disciflora* f. *floccosa* differs only in the short-tomentose, often ferruginous pubescence of the leaves. The two types of trichomes may be mixed in various proportions, so that leaves even on the same plant may differ in pubescence (thus recalling the distinction between *C. stolonifera* and its f. *baileyi*). The validity of the quantitative differences described by Wangerin in proposing his species must be tested by adequate study of the plants in the field.

CORNUS STOLONIFERA f. **flaviramea** (Späth) Rickett, comb. nov. *C. alba* var. *flaviramea* Späth ex Koehne, Mitt. Deuts. Dendr. Ges. **12**: 39. 1903. *C. stolonifera* var. *flaviramea* Rehder in L. H. Bailey, Cycl. Am. Hort. **1**: 377. 1900. Though the yellow-stemmed "red osier" dogwood is known only in cultivation, it may be sufficiently stable to need formal treatment. Obviously the distinction merits nothing more than the rank of forma.

THE NEW YORK BOTANICAL GARDEN
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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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TAXONOMY

(See also under Genetics: **Babcock**; **Darrow & Camp**; under Mycology and Plant Pathology: **Lepage**)

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